

Development of an Index of Sea Turtle Abundance Based Upon In-water Sampling With Trawl Gear

**Final Project Report
to
The National Marine Fisheries Service
National Oceanic and Atmospheric Administration**



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Development of an Index of Sea Turtle Abundance Based Upon In-water Sampling With Trawl Gear

by

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Executive Summary

This study was designed to provide fundamental data for the in-water population of turtles along the southeast coast of the United States, with particular emphasis on measuring abundance. Much of our knowledge of sea turtles has come from data collected on nesting beaches or opportunistically during regulation of fisheries. Through an in-water survey we hoped to provide a better understanding of turtle abundance, spatial variability and population characteristics (e.g., size, sex, life history, genetic composition, health).

The turtle research community has long recognized the need for such data. In 1998 the Turtle Expert Working Group gave voice to the need in calling for further in-water studies. Despite the value of data such a project might produce, the concept of a large-scale in-water survey was regarded as somewhat risky. These concerns were not unfounded. In the past, trawl surveys of similar design caught few turtles. In contrast, recent anecdotal information suggested that numbers of turtles in the water were increasing. To address concern over the possibility of low catches and to assess interactions of turtles with fishermen, the fishery independent sampling design was limited to depths between 15 and 40 feet in order to saturate the area with sampling stations. Additionally a fishery-dependent sampling component using commercial trawlers was added to the project.

Concern over low catch of turtles was quickly dispelled. It appears that the loggerhead turtle population in this study area, as reflected by our data, is much larger than it was in the 1970s and early 1980s. Our catch rates are much higher than those reported for fishery-dependent surveys carried out on commercial shrimp trawlers. Differences in gear and towing speed may account for these higher catch rates, but it appears that loggerheads, at least the juveniles, are indeed more abundant now. Perhaps, this increase in abundance is due, in part, to the mandatory use of turtle excluder devices (TEDs) beginning in 1988 in South Carolina and regionwide in 1990. It may also reflect the rapid growth in nest number for loggerheads on south Florida beaches, in which case, perceptions among shrimp fishermen of an increasing turtle population may be misleading for the northern subpopulation.

Development of a scientifically valid index of abundance for loggerhead turtles was the primary goal of this study. We believe we have been successful in establishing a useful regional index of abundance. The values for the four years of this study range from 0.48 to 0.59 loggerhead turtles per 30.5m-net-hour. Although a majority of our stations (75%) produced no turtle catch, the mean catch rate has been remarkably similar each year giving us confidence that the methods and sampling effort have been adequate to establish a reasonable index of abundance.

Fishery-independent catch rates and shrimp trawler effort were used to estimate the number of interactions between shrimp trawlers and loggerhead turtles in South Carolina waters during the summer (May through August). Estimated total interactions during summer in 2001, 2002, and 2003 were 15,562, 14,311, and 18,625, respectively. These estimates are built on several assumptions that the reader should consider carefully. Despite these assumptions, we feel that the estimates are accurate at least within an order of magnitude.

As we began collecting data, it became clear to us that one simple annual index of abundance may be useful in examining long-term trends in overall turtle population status on a regional basis, but a number of inherent temporal, spatial, and perhaps environmental factors can affect turtle catch rates. We have seen that loggerhead abundance increases at lower latitudes. Inclement weather, for whatever reason, seems to reduce catch rates. These factors need to be recognized when a regional index of abundance is developed.

Over the four years of this study, a disturbing trend of reduced catch rates in the smaller size classes was noted. Examination of annual length frequency plots indicated that growth could account for a shift to larger size classes, but the observed decline in percentages of turtles in the smallest size classes may indicate a recruitment failure, perhaps related to declining nesting activity or an increase in natural mortality rates of smaller juveniles. However, little is known about the process of recruitment from the oceanic to the neritic juvenile stage and therefore numerous alternative explanations are possible. Regardless the reason, this pattern bears continued observation.

It is also clear a mix of individuals from several subpopulations of loggerheads occurs over the range of this study. Given that abundance trends for different subpopulations are possible, it is imperative to segregate turtle catch data by subpopulation. This, however, is no simple matter. Analysis of mitochondrial DNA is ideal for tracing offspring to nesting females and natal beaches; however, there is overlap of at least one haplotype that occurs on nesting beaches throughout the east coast and into the Gulf of Mexico. Therefore, for turtles of that haplotype, one must make assumptions and apply those to a probability analysis when assessing subpopulation trends. These assumptions reduce the robustness of the subpopulation data analysis and leave questions regarding the true population status, particularly for the northern subpopulation. Acknowledging these questions, analysis of DNA data indicated that natal origin for loggerhead turtles captured in this study was 19% (range 14-25%) from the northern subpopulation and 66% (range 60-70%) from the southern subpopulation.

Juvenile turtles exhibited some noteworthy patterns in spatial distribution. We have observed that juveniles may be more closely associated with inlets, perhaps because of more abundant prey, while adults may be more evenly distributed throughout the near-shore coastal area. We have also observed that juveniles, regardless of genetic haplotype, appear to have strong feeding site fidelity as demonstrated by inter-annual tag recaptures that were typically made near the initial tagging and release sites. This feeding site fidelity may underscore the importance of the prey base found in the near-shore areas of the Carolinas and Georgia and is probably a critical aspect of the life history of loggerheads for both east coast subpopulations and perhaps others.

This project significantly improves understanding of turtle health. We provide values for blood chemistry of healthy and sick turtles as a reference for individuals charged with caring for sick turtles. Turtles that were deemed "sick" routinely exhibited blood chemistry values consistent with those of stressed or ill animals. A spin-off study that was facilitated by project-provided blood and scute samples indicated that methylmercury can be relatively high in sea turtles (Day, 2003). Given that this area of the coast is known to be high in methylation rates of mercury and methyl mercury is common in prey items, the use of local feeding sites may jeopardize the health of migratory juveniles. Though sample sizes in the initial study were small, mercury levels

in stranded turtles on SC beaches were found to be significantly higher than those for turtles capture at-sea live. Mercury may impair nervous systems and perhaps alter turtle behavior making those turtle more vulnerable to predators or interactions with man.

Analysis performed North Carolina State University confirmed the presence of fibropapilloma in tissue samples of two loggerhead turtles collected in Georgia waters. Additionally 5-13% percent (depending upon year) of the turtles found in this study had evidence of significant trauma from boat propellers or sharks. Although turtle mortality to shrimp trawlers may be greatly reduced now because of the latest advancements in TEDs, it is clear that juveniles and adults will continue to be directly and indirectly affected by man.

Introduction

Since the 1970's, concern over the plight of sea turtle populations of the United States has increased steadily with much of the concern being based upon apparent declines in sea turtle nesting on beaches and relatively high stranding rates of dead juveniles and adults (Carr, 1972). The vast majority of the turtles nesting and stranding on the Carolinas, Georgia, and northern Florida beaches are loggerheads (*Caretta caretta*). Other sea turtles that are uncommon or rare along the coast are Kemp's ridley (*Lepidochelys kempii*), green (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), and hawksbill (*Eretmochelys imbricata*). In 1975, the Atlantic loggerhead was proposed for listing under the threatened category of the United States Endangered Species Act and listed on 28 June 1978 (Federal Register, Volume 40, Number 98).

Tagging studies of nesting female loggerheads indicate that most return to the same beaches in successive breeding seasons (Bjorndal *et al.*, 1983), and it is widely accepted that females return to their natal region to nest. Stonebruner (1980) concluded that subtle differences in body depth of nesting females indicated that the population of loggerheads on the Atlantic coast may be segregated into two different breeding populations. Caine (1986) found two distinct assemblages of carapace epibionts on nesting loggerheads with the separation occurring on the eastern coast of Florida, also suggesting discrete northern and southern populations of loggerheads. Bowen *et al.* (1993) used mitochondrial DNA to confirm that two separate subpopulations exist -- one in the Carolinas and Georgia (the Northern Nesting Subpopulation) and a second in Florida (the South Florida Nesting Subpopulation). The Turtle Expert Working Group (1998) describes the northern subpopulation as extending into Florida to about 29°N or New Smyrna Beach. The loggerhead Recovery Team is now using Ameila Island, Florida as the southern extent for the northern subpopulation. Bowen *et al.* (1993) noted that the northern subpopulation was likely the result of colonization from the south since the last glacial period 18,000 to 12,000 years ago. Because of nesting fidelity to natal regions and slow gene flow, it is unlikely that an extirpated northern subpopulation would be replenished on a contemporary time scale due to nesting female dispersion.

Sea turtle population assessments have been conducted primarily by counting nesting females or numbers of nests laid on beaches. This sampling provides data on adult females only, and provides no information on abundance of juveniles or adult males. Because there is a lag time of 25-30 years for loggerheads between female hatchlings entering the ocean and first nesting on the beaches, methods that can provide an assessment of relative abundance for juveniles are important tools for detecting population trends that will not be reflected on nesting beaches for many years. The Turtle Expert Working Group noted its top priority for loggerheads is development of long-term, in-water indices of abundance to identify relative abundance of sea turtles:

“Long-term, in water indices of loggerhead abundance in coastal waters are needed to identify relative abundance of sea turtles over time, and to detect changes in size composition with implications regarding recruitment....studies should be in each Sea Turtle Conservation Zone.” (Turtle Expert Working Group, 1998)

A Sea Turtle Conservation Zone, or more correctly, the Atlantic Shrimp Fishery – Sea Turtle Area was established as part of the 1996 TED regulations. It includes all coastal and offshore waters out to 10 nm along the coast of Georgia and South Carolina from the Georgia-Florida border to the South Carolina-North Carolina line (Federal Register 66944).

In 1980, systematic surveys of beach strandings of sea turtles began along the Atlantic coast (Hopkins-Murphy *et al.*, 2001). Circumstantial evidence collected by these new surveys indicated that the timing of sea turtle strandings and shrimp fishery activity were linked – suggesting that shrimp trawling was the likely cause of many if not most of the turtle strandings (NRC, 1990). By 1989, evidence and concern had mounted, resulting in new federal regulations promulgated by the National Marine Fisheries Service that required the use of Turtle Excluder Devices (TEDs) in shrimp trawlers. TED designs were initially variations of an inclined vertical metal grid that directed turtles through exit holes in either the top or bottom of the trawl net, depending upon whether the TED was leaning forward or aft in the net. Soft TEDs constructed entirely of a large-mesh deflector panel sewn into the net were also permitted for use. TEDs were unpopular at the time, with shrimpers complaining about extra purchase and installation costs, safety factors, and potential loss of shrimp.

Initially, TEDs were required in shrimp trawl nets only during the warm-weather seasons, but were required year round in 1991. Through the 1990's, new TED designs were approved, some were subsequently recalled, and all have been modified periodically by regulation. With each new TED regulation, the majority of the fishermen in the shrimping fleet objected and pressed the National Marine Fisheries Service and the states to justify these new regulations. Many shrimpers argued that new regulations were not needed, that the shrimpers were not causing mortalities, and the turtle population was rebounding. Meanwhile, there is mounting concern that nesting activity was declining in South Carolina, raising additional concerns for managers and biologists (Hopkins-Murphy, 2001). The Turtle Expert Working Group noted in 2000 that “No trends are detectable (for nests) for North Carolina, South Carolina or Georgia during that period (1989-1998),” although a longer data set (1975-1998) for Cape Romain National Wildlife Refuge in South Carolina indicated a -2.7% annual change for the long term with most of that change occurring in the 1970's. This growing controversy prompted Dr. Paul Sandifer, then Director of the Marine Resources Division of the South Carolina Department of Natural Resources, to convene a meeting in June 1997 of DNR biologists, NMFS turtle experts and commercial shrimpers. A consensus opinion generated at this meeting was that an in-water study of sea turtles was needed to provide more definitive data on population trends of loggerhead sea turtles in the “South Atlantic” region. Several previous studies examined relative abundance of sea turtles along the Atlantic coast utilizing either observers on active shrimp trawlers or directed operations in localized studies that were usually related to assessing potential impacts to turtles caused by dredging. From this 1997 meeting, South Carolina DNR initiated efforts that eventually led to the present study. This study uses a stratified, random sampling design that should provide unbiased catch rate data for sea turtles, and should presumably target adult males and females during the breeding season, as well as juveniles. The general concept was to establish a scientifically reliable catch-per unit-effort value that could serve as a standard for comparison with similarly collected data in future years.

Henwood and Stuntz (1987) reported sea turtle catch rates observed on shrimp trawlers in the Gulf of Mexico and Atlantic Ocean during the period 1973 through 1978. Observers rode trawlers from North Carolina to Florida, although data collected in the Cape Canaveral channel and adjacent grounds were excluded. In the Atlantic, 453 loggerheads were captured in 9,943 net hours for an overall catch rate of 0.0456 turtles per net hour.

Hillstead et al. (1978) conducted an interview survey of shrimp trawler captains to estimate incidental capture rates of sea turtles by shrimp trawlers off the Georgia coast in 1976. This study produced an estimate that one sea turtle was captured per sixteen trawls (combining catch from multiple nets and having an average tow time of 2.1 hours). Ulrich (1978) led a study in which onboard observers were placed on commercial shrimp trawlers working off the South Carolina coast in 1976 and 1977. In the two years combined, 52 loggerheads were captured in 1,342.2 hours of trawling for a catch rate of 0.0387 turtles per hour (catch from double-rigged nets combined). Monthly averages ranged from 0.014 to 0.061 turtles per hour. Keiser (1976) reported capturing seven loggerhead turtles in 1974 and 1975 while collecting by-catch data on shrimp trawlers off South Carolina, but not enough explanation was given to compute average catch rates. Van Dolah and Maier (1993) reported relatively high catch rates in large-mesh research trawls targeting sea turtles in the Charleston Harbor shipping channel in 1990 and 1991. Each experimental trawl tow was standardized to cover 1,500 m of bottom with tow duration being from 15 to 20 min. each. Conversion of the overall catch rate of 0.125 turtles per sample to hourly catch rates yields values of 0.375 to 0.50 turtles per hour. The present study was designed to concentrate on loggerhead sea turtles with emphasis on the "northern subpopulation" which nests from Amelia island, Florida through the Carolinas. The northern subpopulation is considered to be at risk based upon an apparent long-term decline in annual nesting activity. The northern subpopulation had approximately 7,500 nests in 1998 compared to about 83,400 nests in the South Florida Nesting Subpopulation (Turtle Expert Working Group, 2000). The South Florida subpopulation appears to be increasing in size with an average increase of about 3.6% per year, whereas the northern subpopulation is remaining stable or even showing decline in some locations. Genetic data were collected in this study in an effort to learn more about the occurrence and degree of mixing of these two subpopulations on foraging grounds, as well as occurrence of loggerheads from other subpopulations in the Atlantic Ocean. If there is an increase in the at-sea loggerhead population as suggested by anecdotal observations from fishermen, it is important for managers to understand how much of the increase is likely attributable to the rapidly growing South Florida subpopulation or to other subpopulations.

While the primary objective of this research was to evaluate techniques for establishing a scientifically-valid, in-water index of abundance for sea turtle species, the study also provided a relatively rare opportunity to collect associated data and to collaborate with other researchers who are studying sea turtles or other coincidentally caught species. This study also provided an opportunity to examine genetic make up of individuals, determination of sex for both juveniles and adults, and general health condition of turtles, including observations of traumas caused by interactions with boat propellers and sharks. Collection and analysis of blood samples was used to help validate blood chemistry standards that were based on relatively few turtles. Staff provided tissue

samples to other researchers for analysis of potential toxicants, including mercury, and parasites. By-catch (all fauna other than sea turtles) in the trawls was recorded to provide potential insights into relationships turtles may have with prey and predators, or perhaps to provide information on types of benthic habitats that might be important for sea turtles.

General Methods

The primary method employed in this study was an at-sea fishery-independent sampling effort utilizing research vessels. A much smaller and ancillary effort utilized contracted commercial shrimp trawlers in a fishery-dependent effort.

Fishery-Independent Methods

The fishery-independent effort covered the entire area from Winyah Bay, South Carolina, to St. Augustine, Florida (Figure 1). In each year of the study, a stratified random sampling design was employed. The area was roughly divided into three zones – northern, central and southern – with a vessel assigned to sample each zone. This method facilitated simultaneous sampling throughout the study area, thus minimizing temporal effects on catch rates of sea turtles. The three vessels working each year successfully sampled an average 652 stations per year (range= 602 to 709). All stations were between the depths of 4.8 and 14.9 m (15-40 ft). The northern zone, between Winyah Bay and St. Helena Sound, SC was sampled throughout the study with the R/V *Lady Lisa* which is a 27-m (72 ft) double-rigged St. Augustine shrimp trawler belonging to the South Carolina Department of Natural Resources. The central zone, between St. Helena Sound and St. Catherine's Inlet, GA was sampled with contract shrimp trawlers each year. During the first year, the F/V *Miss Hilda*, a 26-m (70-ft) double-rigged shrimp trawler was used. In the last two years, the F/V *Miss Tina*, a 26-m (70-ft.) double-rigged shrimp trawler, was used. The R/V *Georgia Bulldog*, a 27-m (72 ft) double-rigged shrimp trawler owned and crewed by the University of Georgia Marine Extension Service, sampled the southern zone throughout the study from St. Catherine's Inlet, GA to St. Augustine, FL.

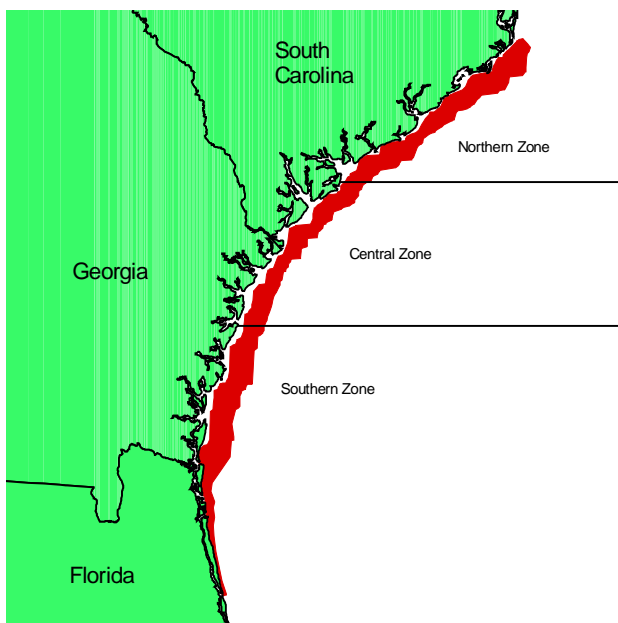


Figure 1. Area sampled in fishery-independent effort (red).

At each station, a thirty-min tow was made with two 20-m (65 ft) flat trawls constructed of 16-cm (8-in.) stretch mesh webbing in the body and 5.1-cm (4-in.) stretch mesh in the tail bag. The trawl doors were 2.44 m x 1.02 m (8 ft x 40 in.) with 1.9-cm (3/4-in.) iron shoes. Tickler chains were measured to be 0.9 m (3 ft) shorter than the footrope. This gear was in accordance with standardized gear as specified by the National Marine Fisheries Service to be used by the vessels contracted throughout the region to remove sea turtles from shipping channels where bottom dredges are operating (Dickerson et. al., 1995).

Bottom trawling time was standardized to 30 minutes on the bottom, with typically 1-2 min to deploy the gear and 2-3 min to retrieve it. Times were recorded for doors submerging, dogged off when on bottom (start time), start of haul back off bottom (end time), and doors at the surface.

A 4.5-m (15 ft) try-net with 1.8-cm (3/4") stretch mesh was fished to obtain abundance data for other co-occurring species. This net was towed for 15 min. bottom time during the first part of the turtle trawl tows. Total number and total weight for each species was recorded. Individual lengths were recorded for selected species (Table 1).

Table 1. List of priority species.

| | |
|---------------------------------|----------------------|
| <i>Penaeus setiferus</i> | white shrimp |
| <i>Penaeus aztecus</i> | brown shrimp |
| <i>Penaeus duorarum</i> | pink shrimp |
| <i>Callinectes sapidus</i> | blue crab |
| <i>Arenarius cribbareus</i> | speckled crab |
| <i>Stomolophus meleagris</i> | cannonball jellyfish |
| <i>Scomberomorus cavalla</i> | king mackerel |
| <i>Scomberomorus maculatus</i> | Spanish mackerel |
| <i>Cynoscion nebulosus</i> | spotted seatrout |
| <i>Cynoscion regalis</i> | weakfish |
| <i>Leiostomus xanthurus</i> | spot |
| <i>Menticirrhus americanus</i> | southern kingfish |
| <i>Micropogonias undulatus</i> | croaker |
| <i>Sciaenops ocellata</i> | red drum |
| <i>Pomatomus saltatrix</i> | bluefish |
| <i>Paralichthys dentatus</i> | summer flounder |
| <i>Paralichthys lethostigma</i> | southern flounder |
| Epinephelinae | groupers |
| Lutjanidae | snappers |
| | Sharks and Rays |

The routine at each station consisted of taking hydrographic and meteorological observations including surface water temperature taken with a bucket and thermometer, and with the ship's transducer reading, when available. Meteorological and sea conditions included wind speed, wind direction, air temperature, percent cloud cover, and sea height/direction.

To ensure full coverage of each geographical area multiple times during the summer, three priority levels were assigned to stations. The first priority level consists of stations that are typically sampled three times yearly by the South Carolina SEAMAP program. By completing these stations first, the entire sampling area was sampled completely at stations "known" to be safe for trawling. (This was done to provide some assurance that an immediate loss of gear due to rough bottom would not jeopardize the study.) The remaining stations were randomly selected using a 1-min. latitude grid over the SEAMAP strata (15-40 ft depth) and choosing a total number of stations proportional to the area covered by each stratum on a mercator projection. Positions and strata codes were determined for these stations. Within each stratum, the odd and even numbered stations were assigned second or third level priority, respectively. The second priority levels were sampled followed by the third level, with few exceptions. This ensured that the entire sampling area was trawled three different times during the summer season. Before the initial cruise, a coin flip determined whether the stratum to be sampled was the northern or southern portion of the sampling zone for each vessel. In subsequent cruises, we sampled alternately to the north and south. The specific sequence of stations within a priority level was chosen to include both offshore and inshore stations in both mornings and afternoons by proceeding in a north to south or south to north direction (decided by coin flip) in a zigzagging fashion. Stations could be relocated within a 0.5 nautical mile area of the station or to be eliminated because of hazardous bottom. Extra stations were allotted within each stratum to allow for possible alternate station selection. Relatively few days were lost to poor sea conditions, although some cruises were curtailed because of mechanical or gear problems.

Fishery-Dependent Methods

For the fishery-dependent effort, trawling was conducted onboard commercial shrimp fishing vessels. Owners with boats meeting project specifications provided bids to the DNR and the fishermen were compensated for the use of their vessel. During summers 2000 through 2003 the vessels used in the Charleston area were the F/V *Winds of Fortune* captained by Mr. Wayne Magwood and the F/V *Bounty* captained by Mr. Toby Saylor. The F/V *Miss Savannah* captained by Mr. George Puterbaugh of Brunswick was used in the Brunswick, GA area in summer 2000. SC DNR and UGA Marine Extension personnel were observers on vessels in South Carolina and Georgia, respectively. In summer 2000, trawling was conducted for 14 days each in the Charleston and Brunswick areas. For the years 2001 to 2003, trawling was conducted off Charleston for 6, 6, and 5 days, respectively.

Shrimpers were allowed to use their standard shrimp trawls with the turtle excluders devices removed. Each vessel towed four 40-foot nets simultaneously. Tows were limited to thirty minutes of bottom time. Otherwise, captains were allowed to tow as they would have fished during normal shrimping activity.

Basic Turtle Workup

For all vessels, each turtle caught and brought aboard was identified and assigned a unique turtle number, the first two letters consisting of the first letter of the genus and the first letter of the specific name. A four-digit, sequential number was assigned, with the first digit coded to the vessel. Turtles were inspected for overall health status and, if

suitable, placed on a specially designed "turtle chair" that facilitated blood collection from the dorsal cervical sinus as described by Owens and Ruiz (1980). Typically, two procedures were followed for blood collection. For loggerheads, 35-ml of blood was drawn with vacutainer tubes for DNA, toxicology, testosterone, hematocrit, total protein and glucose estimation. Additional samples were taken for mass spectrometry (1 ml from 5-ml red top) and for comprehensive reptile profile (5 ml in a green top with lithium heparin). For Kemp's ridley and green turtles, up to 20 ml of blood was drawn for DNA, testosterone, hematocrit, total protein, mass spectrometry, and CBC analyses. Total blood drawn never exceeded 5 % of the body weight.

Eleven standard measurements (Bolten 1999) were recorded for each turtle: Straight-line carapace length (SCLmin, SCLn-t), curved carapace length (CCLmin, CCLn-t), straight-line carapace width (SCW), curved carapace width (CCW), curved plastron width (PW), body depth (BD), straight-line head width (HW), tail length (tip of plastron to tip of extended tail and cloaca to tip of extended tail). Straight-line measurements (cm) were made using a 1m stainless-steel caliper and curved measurements (cm) were made using a nylon tape measure. Body weight (kg) was measured with hanging spring scales while turtles were held in a nylon rope harness.

Sketches were made of the major features of the dorsal and ventral surfaces of each turtle, noting any abnormalities (old or new injuries, barnacles, etc). Turtles were also inspected and scanned for existing tags. Each turtle was photographed next to a placard showing the turtle's identification number and station collection number. When possible, turtles were videotaped. Turtles were tagged with two external Iconel tags in the axillary margin of each anterior flipper, and with one internal PIT (Passive Integrated Transponder) tag subcutaneously on the right shoulder. If previous tags were found to be in suitable condition, new external tags were not added.

Abundance

The fishery-independent portion of the present study sampled thoroughly the area from Georgetown, South Carolina to St. Augustine, Florida in depths of 4.6-12.2m (15-40 ft). This region provides important feeding grounds for neritic juvenile and adult loggerhead turtles. In addition, anecdotal information gathered in this study confirms that at least some mating occurs within the study region.

To efficiently cover the entire sampling area in a reasonably comparable period of time, the study area was divided into three regions along lines of latitude. The northern region included the area from Winyah Bay, SC to St. Helena Sound in southern SC. The central region covered from St. Helena Sound to St. Catherine's Inlet, GA. The southern region included the area from St. Catherine's Inlet, GA to St. Augustine, Florida.

Expressing catch in terms of standardized effort allows legitimate comparison within, and often, between projects. For this study, catch-per-unit-effort (CPUE) was calculated following the methods outlined first by Henwood and Stuntz (1987) and later refined by Jamir (1999). CPUE as used throughout this report is calculated as the ratio of the sum of the number of animals caught to the sum of the effort expended expressed in units of a single 30.5 meter net towed for one hour (30.5m-net-hr). Effort was converted assuming a simple proportional relationship between nets of different lengths or tows of different times. Stated simply, when tow time or net size doubles, the number of turtles expected in the tow doubles. Clearly, fishing efficiency of nets with very different characteristics cannot be expected to conform to this assumption, but we feel this widely accepted method is appropriate for comparing the results of different studies that employ similar gear.

To facilitate the statistical comparison of catch rates within the study, CPUE was calculated for each day a vessel worked. This produced a sample size large enough to be compared statistically while reducing the confounding effects of large numbers of tows with no turtles. This approach results in variable levels of effort. For this reason, effort was controlled in the statistical model along with the factors "region" and "year".

CPUE used for comparison to studies in the literature was calculated adhering strictly to the method described in Jamir (1999). A ratio of the number of turtles to the standardized effort was calculated for tows of interest. Standard error of the CPUE was then calculated and used to define the 95% confidence interval (Jamir, 1999).

Following the methods outlined by Gerrodett and Brandon (2000), the TRENDS Program was used to detect the minimum rate of annual population change detectable by our project and the minimum duration to detect an annual population change of 25%. CPUE and standard deviations, calculated following the method of Henwood and Stuntz, (1987) were used to calculate coefficient of variation (CV). To allow comparison with the extensive table of data in Gerrodett and Brandon (2000) we adopted the same analysis parameters: Type of growth=exponential, Sign=negative, Tails=2, Alpha=0.2, and Power= 0.9.

Results and Discussion

The loggerhead sea turtle was by far the dominant turtle collected during the study. Moderate numbers of Kemp's ridley turtles were also encountered along with a

few green sea turtles. It is our opinion that the habitats sampled in this project were not the main ones used by Kemp's ridley and green sea turtles in our region (see discussion in species composition section). For this reason, thorough analysis is provided only for loggerhead turtles.

Kemp's ridley CPUE

Catch data for Kemp's ridley turtles are summarized in Figure 2. CPUE was 0.0352 Kemp's ridleys per 30.5m-net-hour over the entire effort with no significant annual variation. In comparison, Henwood and Stuntz (1987) reported a rate of 0.0018 Kemp's ridleys per 30.5m-net-hour on shrimp boats during 1979-1981 along the southeast United States. Data from the SEAMAP program also suggest that numbers of Kemp's have been increasing in our region in recent years (SEAMAP-SA Shallow Water Trawl Survey, 2004).

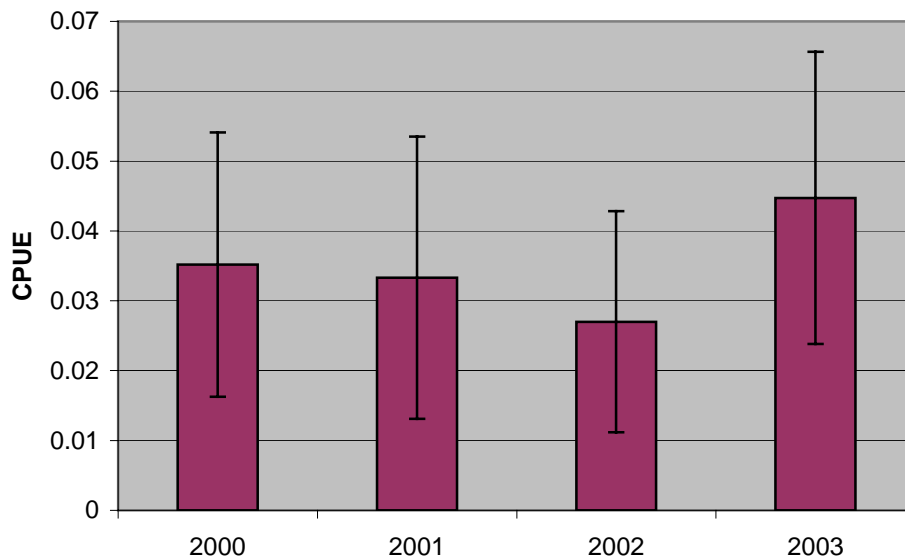


Figure 2. Annual CPUE for Kemp's ridley turtles. Error bars delineate 95% confidence interval.

Loggerhead CPUE: Annual Variation

There was no significant difference for loggerheads in CPUE among years sampled ($P=0.247$). Annual mean CPUE did, however, increase over the study period (Figure 3). The most dramatic increase was observed in 2003 when CPUE approached 0.6 loggerheads per 30.5m-net-hour. While the cause of this higher catch rate cannot be definitively identified based on the data collected, the unique hydrographic and climatic conditions during the summer of 2003 are at least noteworthy.

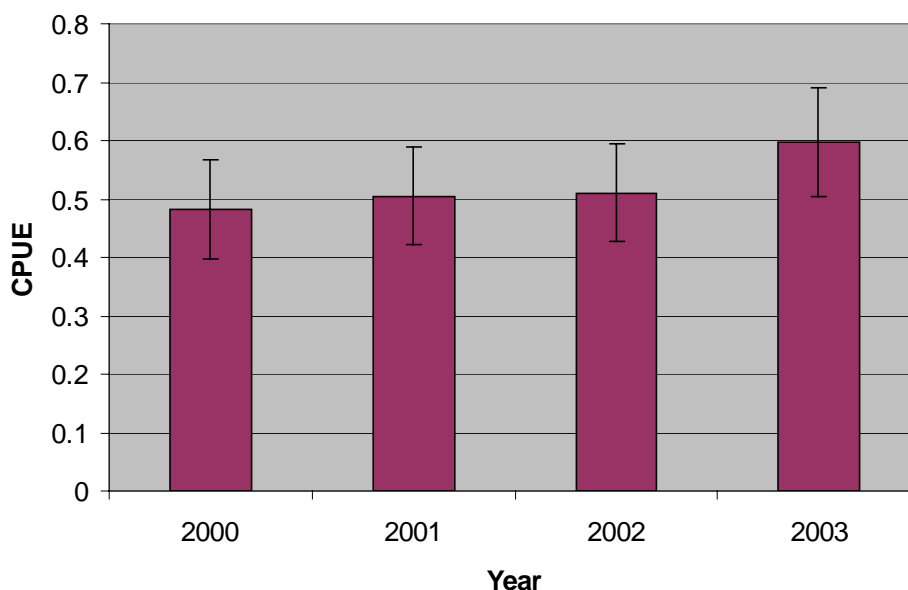


Figure 3. Annual CPUE for loggerhead turtles. Error bars delineate 95% confidence interval.

Beginning in early 2003, the southeastern United States, which had been suffering from a multi-year drought, experienced anomalously high rainfall and a shift of predominant winds to the south. Together, these two factors appear to have resulted in unusual cooling of the continental shelf waters. The phenomenon was first detected along the coast of Florida where the relatively narrow continental shelf provided little barrier between shallow, typically warm inshore waters and deeper cold waters. Consequently, water driven offshore by persistent southerly winds was quickly replaced by colder water moving up the continental slope. The waters cooled progressively northward from Florida and westward from the Gulf Stream, fueled by wind and increased rainfall during the summer and fall. By August a well defined body of cold water from the continental slope overlain by a thin lens of warm surface water had reached 16.5 miles offshore of Charleston SC (Maier, 2003, unpublished data). We suggest that the “squeezing” of warm water into a relatively narrow band along the southeast coast may have caused turtles to move toward the warmer near-shore waters resulting in a concentration of turtles within the region as reflected by the increased CPUEs during summer 2003.

The TRENDS program predicted that change was not detectable within the 4-year sampling period of this project. Gerrodette and Brandon (2000) provide a summary of results of the same analysis applied to 27 sea turtle projects that used various sampling methods (ex. Tangle nets, trawls, aerial surveys). Thirteen of the projects had similar results and were unable to detect changes within the scope of the project. Six of the projects were sufficient to detect annual changes of 2-3%, but all of these were limited in geographic scale.

The second calculation presented by Gerrodette and Brandon (2000) estimated the minimum duration in years to detect an annual change of 25%. The calculation was performed for the projects described above. Among the projects they assessed, the

shortest duration to detect a change was 3 years. The remainder of projects for which a value could be calculated ranged from 4 to 11 years. Five of the projects were unable to detect a 25% change due to high variation in catch. The present project would be able to detect a change in 16 years.

Loggerhead CPUE: Spatial Variation

The region sampled had a highly significant effect on CPUE ($P < 0.001$). CPUE in the southern region was 0.737 loggerhead turtles per 30.5m-net-hour compared with 0.395 and 0.392 for the central and northern regions, respectively (Figure 4). The Bonferroni post hoc test showed that the CPUE in the southern region was significantly higher than both regions to the north. The SEAMAP program samples a similar geographic range and also reported higher densities of loggerhead turtles in the southern portion of the south Atlantic Bight, particularly off Georgia and Florida (SEAMAP-SA Shallow Water Trawl Survey, 2004).

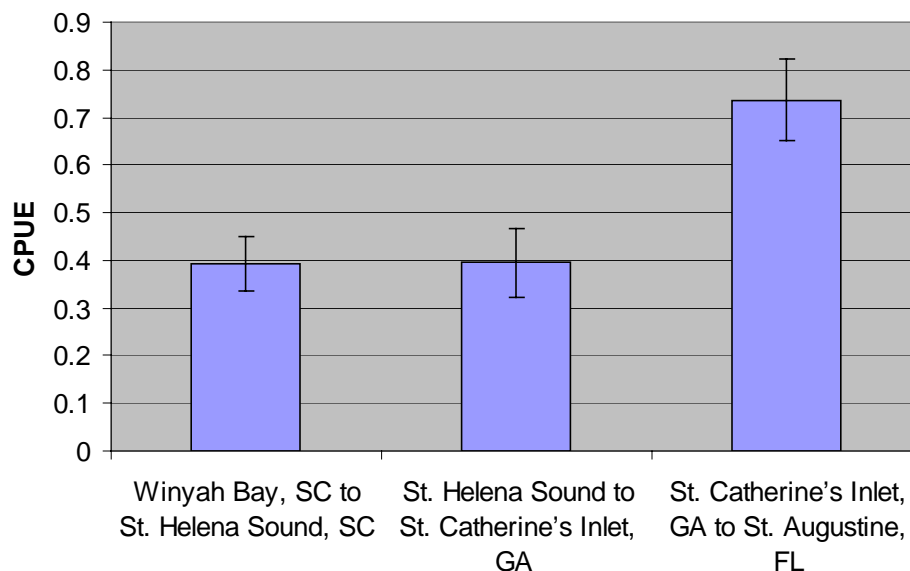


Figure 4. Catch rate of loggerhead turtles by region. Error bars define the 95% confidence interval.

CPUE of loggerhead turtles in the present study, collectively or by region, were remarkably high compared to values reported in the literature. Moreover, the only studies we could find with comparable or greater catch rates involved sampling efforts conducted on relatively small spatial or temporal scales. Schmid (1995), sampling near Cape Canaveral reported monthly June and July catch rates for 1989 through 1991 ranging from 0.0568 to 0.6988 loggerhead turtles per 30.5m-net-hour with a median value of 0.1777. Even in this case, where the effort was focused in an area known for concentrations of loggerheads (Butler *et al.*, 1987), none of the values exceeded that calculated for the southern region of our study. Bolten *et al.* (1994) and Henwood (1987) calculated catch rates that overlap or exceed the present study, but like Schmid (1995), their effort was concentrated in the turtle-rich Cape Canaveral Ship Channel. Conversion of data from Van Dolah and Maier (1993) to the standard CPUE units used in our study,

yields a value of 0.35 loggerhead turtles per 30.5m-net-hour which is less than the value for the northern region in the present study (0.392). However, this effort was confined to the Charleston Harbor Shipping Channel, which they suggested possesses larger concentrations of turtles relative to the surrounding, shallower areas.

Because large-scale at-sea turtle sampling projects are expensive, labor intensive, and logistically difficult, relatively few have been conducted. Bullis and Drummond (undated NOAA publication) summarized turtle catches from exploratory tows conducted in depths of less than 50 fathoms between 1950 and 1976. They reported a catch rate of 0.009 turtles per hour (29 turtles in 2955 hours of towing) using nets with a minimum headrope length of 60 feet. This catch rate converts to 0.015 loggerhead turtles per 30.5m-net-hour, which is substantially lower than the present study. Beatty et al. (1992) report on incidental catches of loggerhead turtles in the SEAMAP groundfish survey off the southeastern United States. For summer cruises, they report a CPUE of 0.043 loggerheads, which is equivalent to 0.028 loggerhead turtles per 30.5m-net-hour.

Observer programs of commercial shrimp fisheries provide another potential source of turtle catch data suitable for comparison to the present study. However, these data are also spatially biased in that the areas favored by shrimp trawlers are not distributed randomly and may also be favored feeding areas of turtles. An additional complicating factor is that nets used in the present study employed large mesh and towing speeds faster than those typically employed by shrimp trawlers. Nevertheless, data from the fishery-dependent portion of the present study produced catch rates equal to or higher than those found in the fishery-independent sampling. Based on data collected aboard shrimp vessels in the Atlantic from 1979 through 1981 Henwood and Stuntz (1987) reported a CPUE of 0.0456 loggerhead turtles 30.5m-net-hour. This value was later corrected by Jamir (1999) to 0.04791, but the catch rate still remains approximately an order of magnitude lower than the present study. Ulrich (1978) performed a similar survey aboard shrimp boats in South Carolina in 1976-1977. He reported the capture of 52 loggerhead turtles during 1343.1 hours of trawling aboard double-rigged vessels fishing nets ranging from 55-90 ft. These data can be used to calculate a maximum catch rate standardized to the effort of a 30.5 m-net-hour by assuming all vessels used the smallest (55-ft) net. Calculated in this manner, Ulrich's CPUE is 0.03871 loggerhead turtles per 30.5m-net-hour. The catch rates of both observer studies are similar, and probably represent a good measure of in-water turtle populations during the 1970s and early 1980s.

Gears compared previously were similar in configuration to those employed in the present study with the exception of mesh size. The gear used in this project had a 4-inch bar mesh that is larger than nets used in commercial shrimping. While we feel that this difference would not cause differences on the order of magnitude observed here, we provide further evidence by comparing large mesh gear used elsewhere to small mesh gear used in the fishery dependent portion of the present study. Van Dolah and Maier (1993) used the same large-mesh gear as the present project and worked in an area of the Charleston Channel sampled in the fishery-dependent portion of the present project. The highest monthly catch rate they report for the comparable season was 0.441 loggerhead turtles per 30.5m-net-hour (calculated from mean of June and July catch rates). It should be noted that this number represents a balanced sampling of the channel in which offshore sections with much higher catch rates were sampled equally to poorly populated

inshore sections. Sampling in the same location aboard the F/V *Winds of Fortune* in the present project, found a similar pattern in spatial distribution to that reported in Van Dolah and Maier (1993), but did not sample the channel equally. Channel sampling of the present study disproportionately sampled from the inshore sections (81%), which were found in both studies to be relatively poorly populated with turtles. Despite the fact that the present study underrepresented the turtle-rich portions of the channel in their sampling, the catch rate was 0.714 loggerhead turtles per 30.5 m net-hour, considerable higher than that reported in the 1991 study. In addition, the offshore section of the channel sampled in this project averaged over 2 loggerhead turtles per 30.5m-net-hour, far beyond anything reported in the 1993 study. This comparison reinforces the conclusion that catch rates of the present study are significantly higher than those presented previously.

Comparison of loggerhead catch data from the present study with historical values suggests that in-water populations of loggerhead sea turtles along the southeastern United States appear to be larger, possibly an order of magnitude higher, than they were 25 years ago. We find it noteworthy also that CPUE of the present study is largely comparable, and often exceeds, those reported historically for areas known to host large aggregations of turtles. Further support for the conclusion of increasing abundance of in-water loggerhead populations comes from SEAMAP long-term data. The continuing South Atlantic SEAMAP project has trawled stations along the southeast coast of the United States since 1989. Although catch rates for the SEAMAP project were considerably lower in the early 1990s they have increased substantially over time ($r^2 = 0.708$; Figure 5).

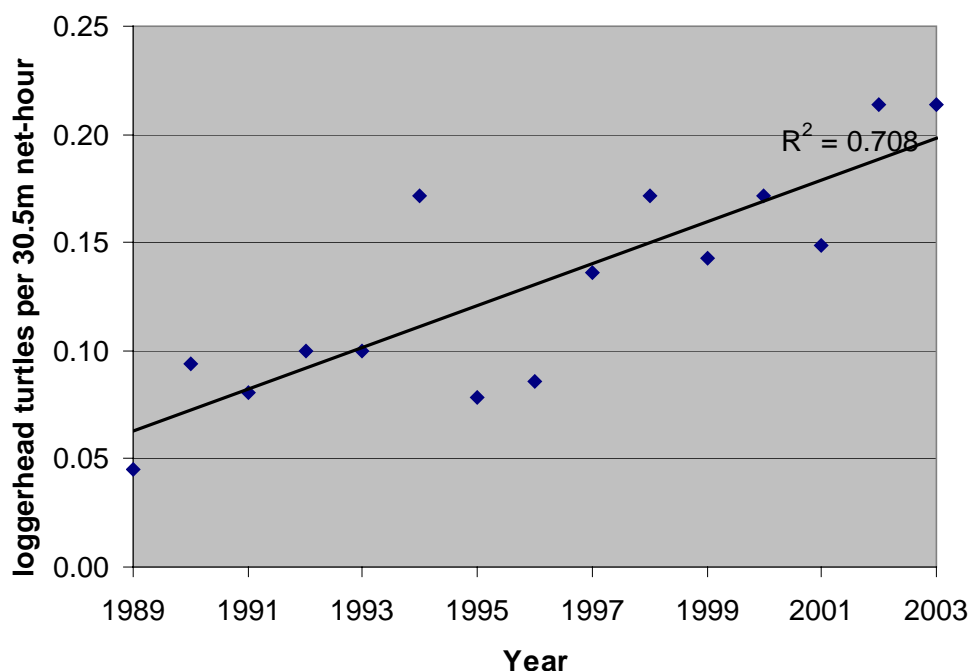


Figure 5. Loggerhead turtle catch rates observed by the SEAMAP program.

In contrast, the finding of increased sea turtles in the water is not supported by the trend for nesting females in the northern subpopulation which has been basically unchanged or showing slight declines since 1990. If turtle abundance in the water is indeed increasing, this should be manifested by an increase in nesting females as these turtles become mature. Alternatively, a substantial portion of the increase in turtles in the water may be the result of transient juvenile turtles from south Florida beaches where there has been a long and sustained increase in annual turtle nests.

Population Estimate: Area Swept

An estimate of the total number of loggerhead turtles occupying the sampling area was calculated using the "area swept" method. Since differences in catch rates were detected among regions, calculations were performed by region, and then summed. Mean vessel speed and total tow time were used to calculate the total distance towed. The effective width of the net when fishing, which is 12 m (Dickerson *et al.*, 1995), was then multiplied by distance towed to calculate the total area sampled. The area sampled, divided by the number of loggerhead turtles caught, provided an average area per turtle. The total area of each sampling zone was calculated using ArcView® GIS. An estimate of the number of loggerhead turtles within each zone was then calculated by dividing the total sampling area by the average area per turtle. Estimates for each zone were then summed to provide an estimate of the total loggerheads occupying the area at any time during the sampling effort.

We estimate that on average 26,538 loggerhead turtles occupy the sampling area during June and July. TEWG (1998) estimated the number of loggerhead turtles in near-shore waters of the southeast United States with a "ratio method" that used the number of nesting adults and strandings data. This method yielded a mean of 23,366 loggerhead turtles for the years 1989-1994, which was remarkably similar to our estimate of 26,538 loggerheads.

Fishery Dependent Sampling

Fishery-dependent sampling was originally included as a hedge against the possibility that random sampling of fishery-independent effort would produce few turtles and as a way of getting the commercial fishing industry to “buy in” to the study. Fishery-dependent sampling proved to be a reasonable method to catch turtles, although over the course of the project the fishery-dependent portion was deemphasized because of budget limitations. Because this effort involved daily instead of 5-day cruises, it was a valuable means for collecting relatively large numbers of perishable samples such as fresh blood for chemical and immunological analysis.

Because the sampling was conducted as defined by the fishermen, turtles collected in this effort provide an excellent opportunity to assess the interaction of turtles with shrimping gear.

Results and Discussion

Details of sampling effort are included in the General Methods section of this document. It is noteworthy that catch rates varied significantly among years and locations (Figure 6). It was evident that the relatively small temporal window during which sampling was conducted contributed to high variability in catch rates among years. Also, it appeared that significant spatial differences in abundance of loggerhead turtles, even within a region, contributed to the variability. In the entrance channel to the Charleston Harbor for example, certain sections of the channel had extremely high catch rates while other sections of the channel within a kilometer produced consistently lower catches.

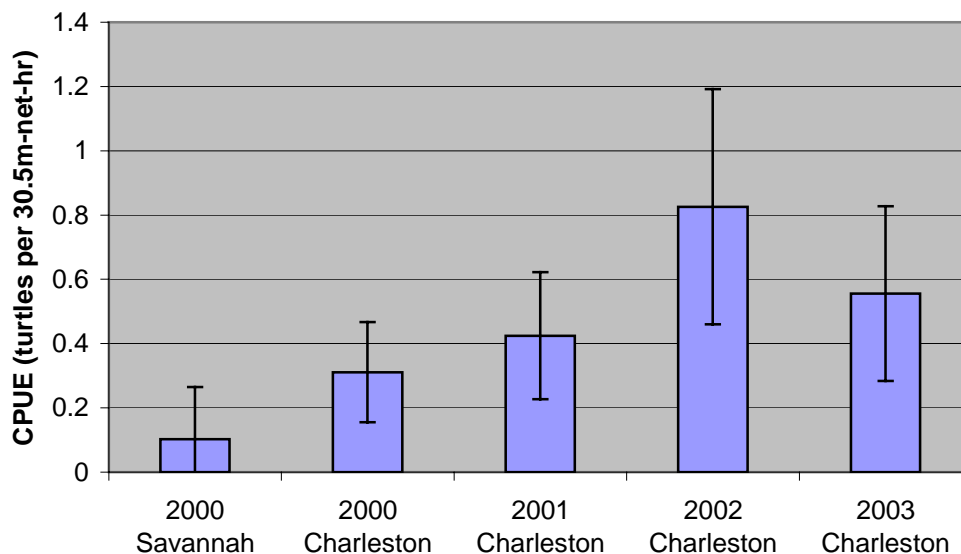


Figure 6. Catch rate of loggerhead turtles in fishery-dependent sampling. Error bars represent 95% confidence interval.

A total of 386 30-minute tows captured 131 sea turtles. Species composition was similar to that of the fishery-independent effort with loggerhead turtles comprising 91% of the sea turtle catch. A total of 11 Kemp's ridleys and one green sea turtle were collected.

Catch rates on the boats working in the vicinity of Charleston were generally higher than the fishery-independent vessel working near this area. However, high variability in catch rates on the fishery-dependent vessel prevented detection of statistically significant differences, except during 2002 when fishery dependent catch rates were extraordinarily high. The greatest number of turtles in one tow during the entire study was eight collected by a shrimp trawler in the Charleston Harbor Channel.

Interestingly, loggerhead turtles caught in the fishery-dependent effort were significantly smaller than those caught in the fishery-independent sampling in the same general area ($P < 0.001$, Mann Whitney Rank Sum Test). Median length was 61.6cm compared with 66.7 cm in the fishery-independent sampling. Only one turtle was greater than 77 cm SCL. The single adult captured was a female bearing tags applied when it nested on Cumberland Island, Georgia. The F/V *Miss Savannah* working near Cumberland Island in 2000 caught it.

The sex ratio for fishery-dependent turtles was 3.05 females to 1 male. This ratio is higher than that reported for the fishery-independent effort, which is likely due to the relatively small size of the fishery-dependent turtles.

Turtle Population Description

Sea turtles found on the continental shelf of the southeastern United States represent an amalgam of species and life-history stages. Each is tied, with varying degree, to the production of the shallow estuaries and near-shore waters. Herbivorous green sea turtles are closely tied to the estuaries by the algae and eelgrass these shallow environments provide (Burke *et al.*, 1992). In contrast, the loggerhead turtle is known to range over the entire continental shelf (Snover *et al.*, 2000), although greatest numbers are found in depths less than 60 meters (Shoop and Kenney, 1992). Nesting on the beaches of the southeast United States accounts for 35-40% of the worldwide nesting for this species (Ross, 1982). This concentration of nesting activity is reflected in the relative abundance of loggerhead turtles in the adjacent near-shore waters.

The proximity of nesting and foraging grounds along the east coast of the US insures the presence of multiple life stages in the region. Adult female loggerhead turtles are present at least during the months that they are nesting. During these times, they utilize the nesting beaches and the near-shore habitats (Murphy and Hopkins, 1981). Oceanic juveniles recruit to this population at about 52 cm SCL (Snover *et al.*, 2000), presumably from the eastern Atlantic where animals provide the missing size classes between hatchlings and neritic juveniles (Hopkins-Murphy *et al.*, 1999). Emerging evidence from satellite telemetry studies suggests that some of the smaller loggerhead turtles caught off the coast of the southeastern United States may return to the pelagic environment (Bolten, 2000) suggesting that at least some individuals may exhibit a transitional phase between oceanic juveniles and neritic juveniles.

Genetic evidence suggests that the southeastern coast of the United States contains two nesting populations of loggerhead sea turtles (Bowen *et al.*, 1994; Encalada *et al.*, 1998). The south Florida subpopulation is large with approximately 64,000 nests. In contrast, the Northern subpopulation, extending from northeast Florida through North Carolina, has only 6,200 nests (Hopkins-Murphy *et al.*, 1999). It is generally believed that the northern subpopulation, which diverged relatively recently, is vulnerable to extirpation. Moreover, Bowen *et al.* (1994) contend that recolonization would not occur on contemporary time scales. It is estimated that this subpopulation would take more than 1000 years to rebuild (TEWG, 1998).

Methods

Details of fishery-independent sampling protocols and gear used to collect the data described here are covered in the General Methods section of this document. Briefly, sea turtles were collected from three identically outfitted trawlers of similar characteristics. The survey was conducted within the shallow near-shore waters of the southeastern United States utilizing a random sampling design. The effort was prosecuted over a relatively short period (approximately 2 months) during the peak of turtle abundance during summers 2000 through 2003. Sex was determined using radioimmunoassay techniques described by Owens *et al.* (1978).

Results and Discussion

Species Composition

Loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*) and green sea turtles (*Chelonia mydas*) were captured during this study. Each species was present in all regions, though noteworthy trends in regional abundance were observed. The loggerhead turtle was by far the most common, accounting for 93.0% of the sea turtles collected. The Kemp's ridley was second most common accounting for 6.2% of the turtle catch. The green sea turtle (*Chelonia mydas*) was rarely caught averaging 0.79% of the catch, but was distributed evenly among regions.

The Pearson chi-square statistic was used to determine if the proportions of turtle species were significantly different among years and regions. Initial analyses included all turtles species caught, but the results were suspect due to overall low occurrence of green sea turtles and the resulting large number of vessel/year combinations during which no green sea turtles were caught. For this reason, green sea turtles were dropped from the dataset and the data were reanalyzed. Analysis showed that the relative proportion of loggerhead and Kemp's ridley turtles was not significantly different among years ($P=0.826$). However, species composition differed significantly among regions ($P=0.017$). Table 2 illustrates that the difference among regions stemmed from a relatively large proportion of Kemp's ridley in the catch of the vessel sampling the central zone.

Table 2. Species composition as percentages by region.

| | Loggerhead | Kemp's Ridley | Green |
|----------|------------|---------------|-------|
| Northern | 94.9 | 4.3 | 0.9 |
| Central | 88.1 | 10.7 | 1.1 |
| Southern | 93.9 | 5.5 | 0.6 |
| Overall | 93.0 | 6.2 | 0.8 |

The pattern of occurrence of Kemp's ridley turtles among regions poses an interesting, if somewhat perplexing question. An immediate question that must be answered is whether the apparent higher proportion of Kemp's caught on the central region is really a consequence of lower proportion of loggerheads. This can be answered by comparing loggerhead catches between the northern and central region. They are similar (see Abundance section), so this is not the case. CPUE was also highest for Kemp's ridleys in the central region (0.048 turtles per 30.5 m-net-hour). Additionally, it is unlikely that Kemp's ridleys are subject to some environmental stress affecting their distribution across our sampling area as they are known as common visitors as far north as New York (Morreale and Standora, 1993). However, Kemp's are known to occupy estuaries in areas where they feed preferentially on certain species of crab (Morreale and Standora, 1993). It is possible that the relatively large open sounds of southern South Carolina and northern Georgia create a particular enticement in food and/or habitat to Kemp's ridley turtles.

Green sea turtles were rarely encountered during this survey. The few caught were small juveniles. Other in-water studies working near-shore areas have found this to

be the case also. Henwood and Ogren (1987) believed that green sea turtles represented an itinerant population in the Canaveral Ship Channel, tending to congregate around inshore structures such as jetties. Similarly, researchers working in shallow estuarine habitats of South Carolina have found small green turtles to be the most common sea turtle encountered (W.A. Roumillat, pers. comm.). Green sea turtles feed preferentially on marine algae and eelgrass when present (Burke, 1992), which may be the main factor influencing their apparent preference for shallow estuarine habitats over near-shore habitats. Regardless of the reason, it seems likely that their lack of representation in our samples stems, at least in part, from their preference for estuarine habitats not sampled by this project. Green sea turtles collected by this study likely represent individuals passing through the area while moving to, or from, preferred estuarine habitats.

The loggerhead turtle was by far the most common sea turtle encountered in this study. Likewise, loggerhead turtles are known from numerous studies to be the most common sea turtle in the neritic waters of the southeast United States (Ulrich, 1978; Henwood and Stuntz, 1987; Schmid, 1995; SEAMAP-SA Shallow Water Trawl Survey, 2004; Bullis and Drummond, undated).

Sex Ratio

Results of tests to determine sex of loggerhead turtles caught in 2003 were not available at the time this report was prepared. Therefore, data are presented only for loggerheads collected during the 2000-2002 sampling seasons.

Pearson's chi-square statistic showed that there was no significant difference in overall sex ratio among years ($P=0.584$) or regions ($P=0.959$). The overall sex ratio for the project was 1 male to 2.30 females. This is similar to the ratio of 1:2 reported by Owens (1997) for loggerhead turtles collected along the eastern United States from Virginia to Florida Bay. Wibbles et al. (1987) reported a similar ratio of 1:1.9 for live-captured loggerhead turtles along the Atlantic coast.

Despite this consistency in sex ratios among studies it should be noted that at least two lifestages (i.e. neritic juvenile and adults) are combined to compute this ratio. Along the east coast of the United States loggerhead turtles remain in the neritic juvenile life stage for approximately 15 years (Isley, unpublished data). Adults emerging from this stage are very different both physiologically and behaviorally from the 45-50 cm animals that recruited there ~15 years prior. These behavioral and physiological changes occur in the population over a range of sizes. Therefore, it is important to examine data such as sex ratios across the range of sizes collected.

Sex ratios were calculated for each 10 cm size class. Sex ratio of loggerhead turtles caught in this study varied dramatically with length (Figure 7). The size class of first recruits to this population (50-60 cm) has a sex ratio of 1 male to 3.64 females. As size increases, the turtles exhibit a more equal ratio ultimately reaching a ratio of 1 male to 1.14 females for the 90-100 cm size class.

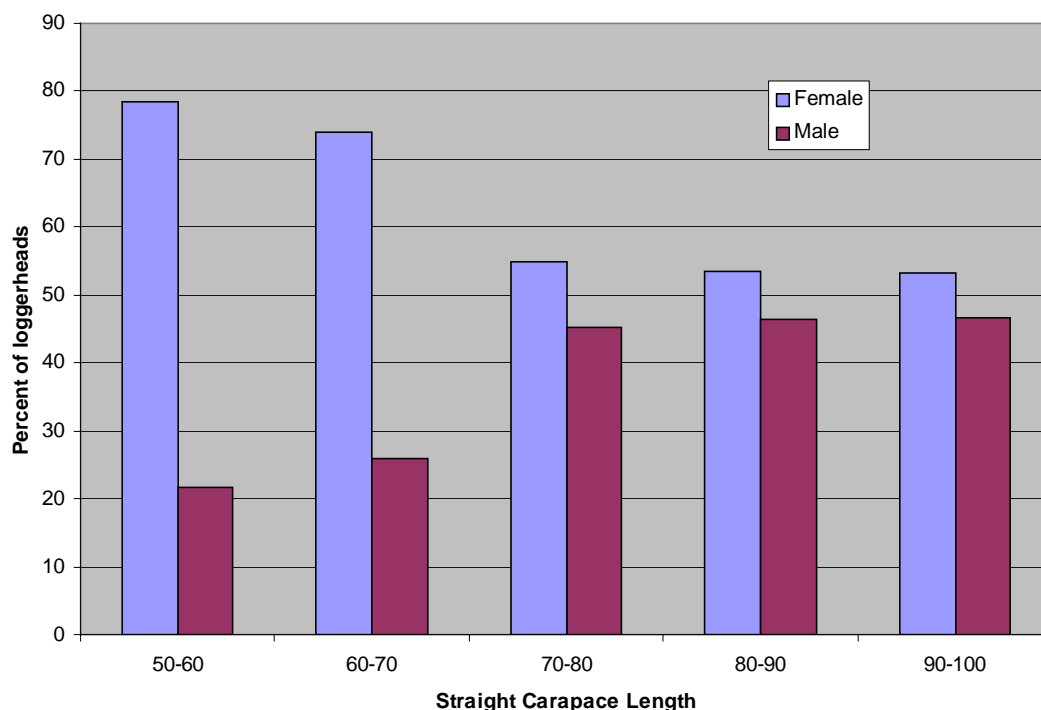


Figure 7. Sex ratio of loggerhead turtles by length.

The observed change in sex ratio may be simply a consequence of preference of maturing loggerhead turtles for areas near their natal regions. The area we sampled was primarily adjacent to nesting beaches of the Northern subpopulation. New recruits to the Carolinas and Georgia may be a mixture of juveniles from multiple subpopulations, but as they begin to mature they may move to areas nearer their natal regions. This explanation would account for the shift we observe from a low ratio of males to females indicative of southern nesting beaches toward a more equal ratio seen in hatchlings from nesting beaches of the northern subpopulation (Mrosovsky *et al.*, 1984).

Genetic studies in the Charleston Harbor channel (Sears *et al.*, 1994), off Georgia (Sears *et al.*, 1995) and in the Chesapeake Bay (Norrgard, 1995) conclude that the composition of in-water loggerheads is composed of an equal proportion from South Florida and the Northern nesting subpopulations. Using this proportion and the subpopulation hatchling sex ratio provided by Hopkins-Murphy *et al.* (2003), we can predict an overall sex ratio for the in-water population of 1 male to 2.33 females (mixture of 50% 9:1, 50% 1:1). Since these studies sampled in-water populations of similar composition to those tested here, their predicted sex ratio of 1 male to 2.33 females is most appropriately compared to our overall measured sex ratio of 1 male to 2.30 females.

While we stress that conclusions about genetic makeup of the in-water loggerhead population are best drawn by direct examination of genetic data, we feel the indirect evidence presented here compels reexamination of existing data for changes in genetic composition among size classes. The fact that changes in genetic ratios might be predicted based on what we know of the life history of the loggerhead turtle only bolsters

the argument. Indeed, natal homing is well documented for female loggerhead turtles, however it may be size dependant in juveniles.

Length

Kemp's ridley turtles ranged in length from 27.1 to 62.5 cm SCL. Kemp's are generally accepted to mature at approximately 65 cm SCL (Zug *et al.*, 1995), so virtually all were immature.

Combined strandings data for the Gulf of Mexico and the southeastern United States for 1996-1997 include animals of the size range reported here (TWEAG, 2000). Those data also include many more small turtles with 50% measuring less than 40 cm. The unusual shape of the size distribution in this study (Figure 8) and the presence of these smaller size classes in the strandings data suggest there may be a size-related habitat preference which may have resulted in smaller turtles not being in the areas sampled by this study.

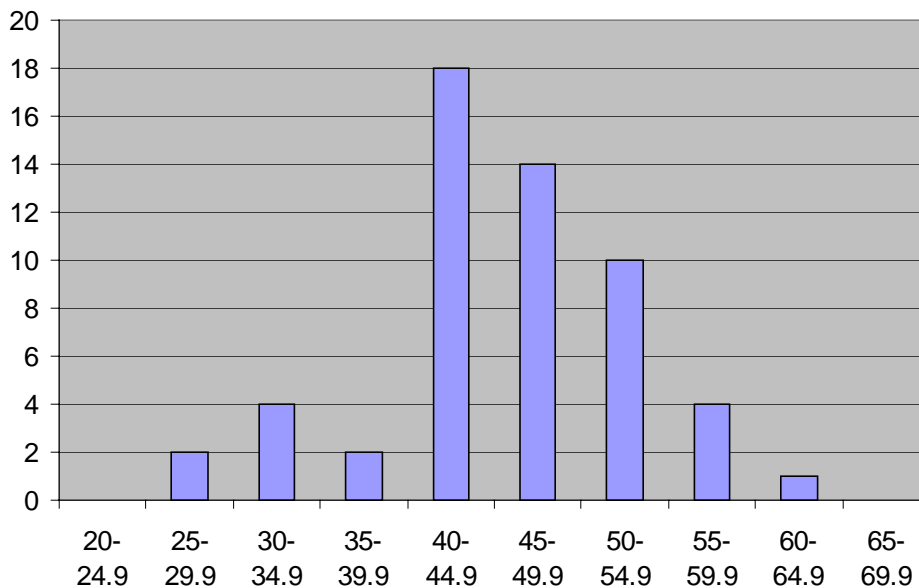


Figure 8. Length-frequency of fishery-independent Kemp's ridley turtles (cm). N=53.

Loggerhead turtles caught belonged predominantly to the neritic juvenile lifestage. TEWG (1998) suggest that 92 cm SCL is a reasonable estimate of first maturity for loggerhead turtles based on data from nesting beaches. Hopkins-Murphy (pers. comm.) believes that that a slightly smaller size, 91cm CCL, provides a better measure of first maturity. Clearly, any population of animals will mature over a range of sizes and for the turtles inhabiting the near-shore waters of the southeastern United States, the range will encompass both of these values. For this reason, we have chosen to present the estimated percentage of juvenile turtles in our study as a range defined by these two estimates. Using this approach, we estimate that between 93.7% and 97.0 % of our loggerhead turtles were immature. This value is similar the 91% juveniles reported for 1991-1998 at the St. Lucie Power Plant on the east coast of Florida (TEWG 2000).

Similarly, 90% of the animals stranding along the southeast coast from 1984-1994 were immature (TEWG, 1998).

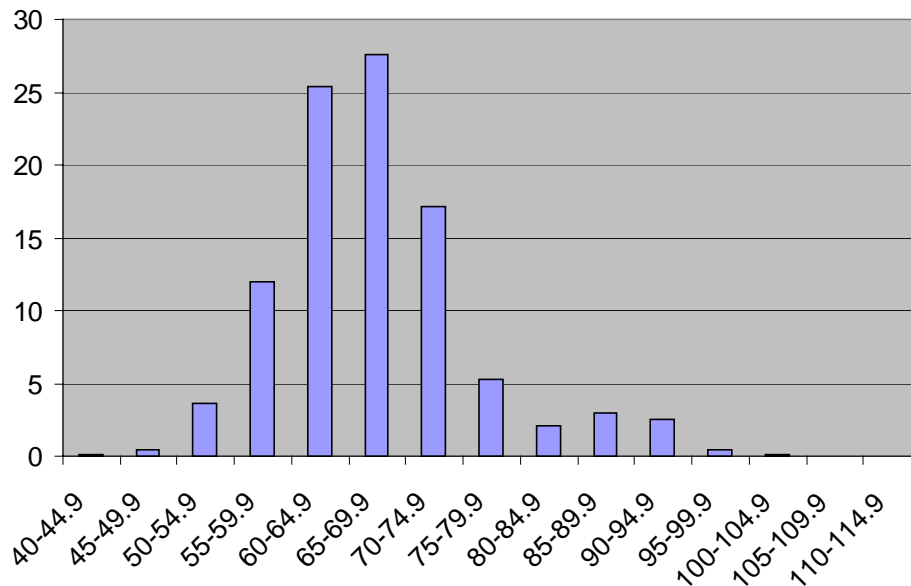


Figure 9. Length-frequency (percent) of loggerhead turtles caught in the fishery-independent portion of the project. Lengths are in centimeters.

A two-way ANOVA was used to assess whether significant differences in length of turtles existed among regions and years. Comparisons were made using straight carapace length (notch-notch), the most conservative measure taken, which also had the advantage of not being affected by damage to the tip of the pygal bone.

Loggerheads caught in the three regions were significantly different in length ($P < 0.001$). Mean turtle length was greatest in the northern region and smallest in the southern region (Figure 10). Bonferroni's post hoc test showed that the turtles from the northern and central region were not significantly different in size, but turtles caught by the vessel working the southern region were significantly smaller than those from the other regions.

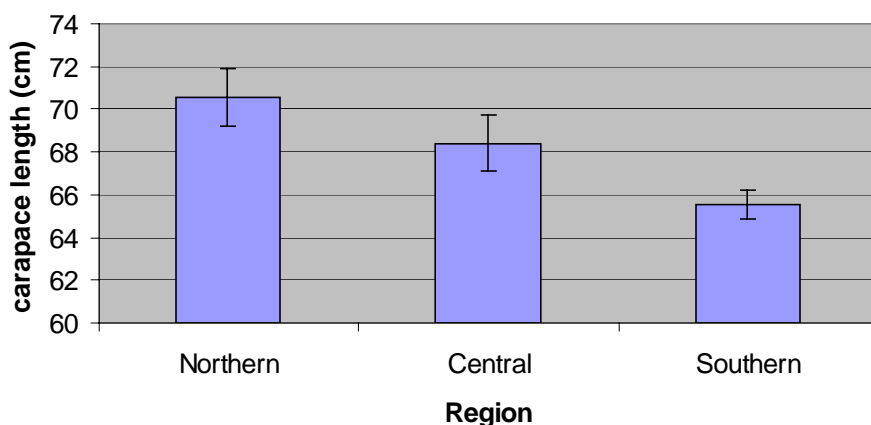


Figure 10. Mean length of loggerhead turtles by region. Error bars show 95% confidence interval.

Though comparison of mean turtle length among year suggested a trend of increasing size, only the years 2000 and 2003 were significantly different from each other ($P=0.002$). Years 2001 and 2002 were not significantly different from each other or other years (Figure 11).

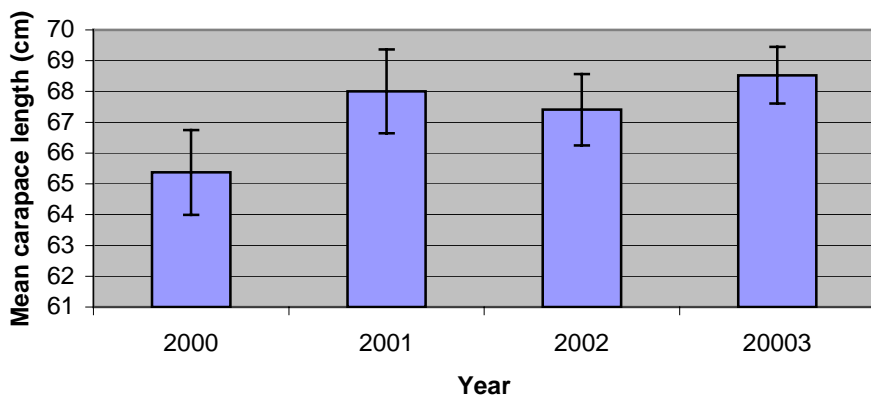


Figure 11. Mean length of loggerhead turtles by year. Error bars show 95% confidence interval.

There are numerous reasons that might account for the apparent increase in the population's mean turtle size over time. Factors include changes in behavior related to immigration/emigration, selective natural or fishing-related mortality, and recruitment failure. In an attempt to understand why the change in mean size is occurring, the data were partitioned by year for more detailed examination (Figure 12). There appears to be an annual shift to the right (toward larger size classes) of the mode. This shift may be accounted for by growth, although there appears to be an absence of new recruits to the

smallest size classes.

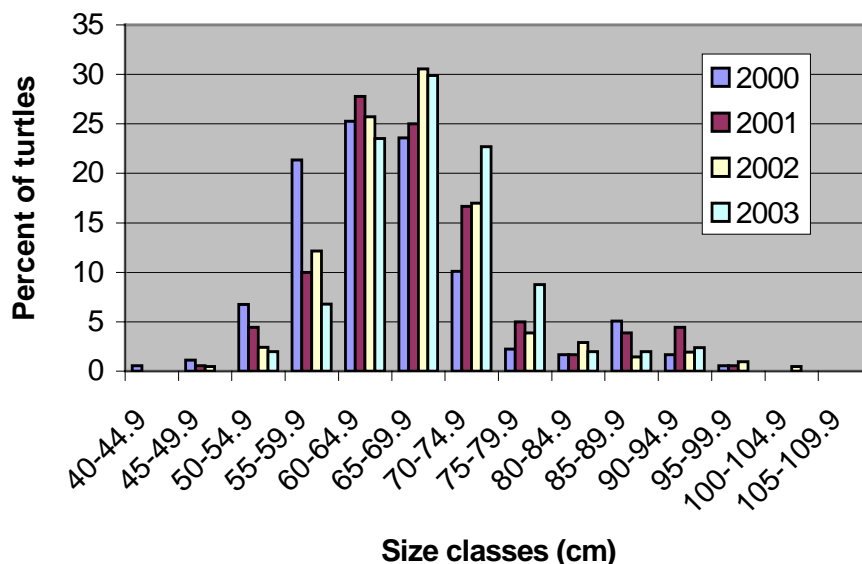


Figure 12. Length-frequency (percent) of loggerhead turtles

In Figure 13 the actual measurements of turtles caught in 2003 are compared to lengths projected from measurements taken in 2000 using a growth rate that was computed from data collected in this study (Isley, unpublished data). It appears that the overall curves are similar for the projected and measured data. In both, the loggerheads in the smallest size classes have disappeared and the mode has shifted to the right one size class. Comparison of these data suggests that growth provides an adequate and simple explanation for the change in the observed change in size of loggerhead turtles.

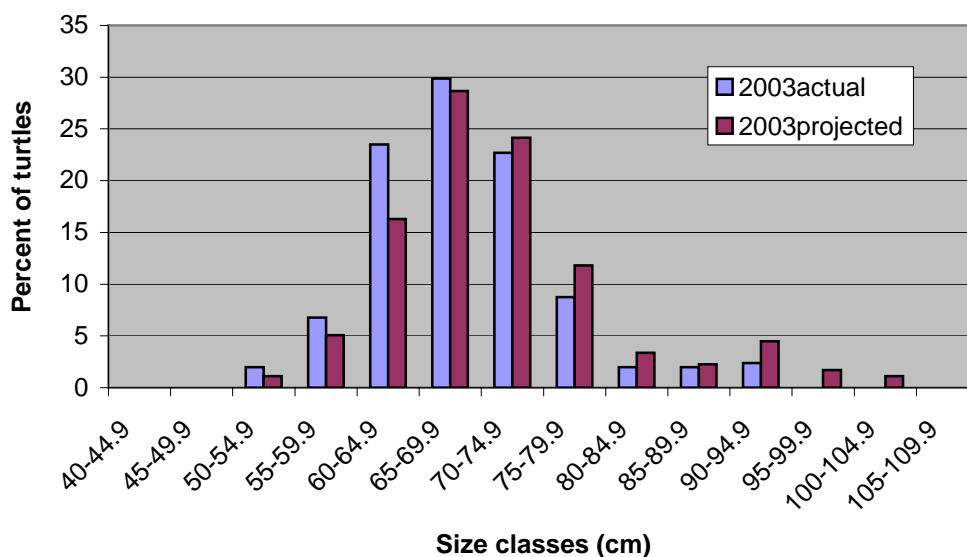


Figure 13. Comparison of observed and projected lengths of loggerhead turtles in 2003.

The disappearance of turtles from the smallest size classes during the course of the project is a phenomenon worth highlighting. If we assume a constant supply of hatchlings and constant natural mortality rates of oceanic juveniles, we would expect a steady recruitment of young turtles to the population of neritic juvenile turtles. One might expect failure of a year class to anomalous conditions during the nesting season, but the data presented in Figure 12 suggest the decline in smaller turtles may be a sustained condition for at least three years of this study. If this absence of new recruits is real and sustained, it suggests that some longer-term condition may be affecting the population. Although some decline in nesting activity has been noted for the northern subpopulation, there has not been any dramatic decline that might result in recruitment failure. Additionally, we know from DNA haplotyping that a significant portion of the juveniles in the study zone is from the south Florida nesting subpopulation that has been growing consistently for some time. Assuming we are seeing a real trend in decline of relative abundance of the smaller turtles, we can only speculate as to possible causes. During much of this study, a prolonged and historic drought afflicted the southeastern United States resulting in reduced river discharge and higher salinities in coastal estuaries. These physical conditions could have altered behavioral patterns of loggerheads or their food resources. Perhaps the unusually high salinities in estuaries opened more suitable habitat for foraging that was outside the areas sampled in this study. However, in 2003 rainfall and salinities were near normal, and no young turtles appeared in the catches.

Among other possibilities, it is possible that some shift in predator abundance or behavior could have resulted in increased mortality rates of hatchlings entering the ocean or while at sea. Some shark species populations, particularly some of the small coastal sharks, appear to have rebounded and could be responsible for increased predation on turtle hatchlings. Over the last 15 years, new federal shark management plans with commercial shark quotas and recreational creel limits have been put into place. Additionally, both the prohibition of commercial fishing nets within a mile of Florida's east coast beaches and the mandated use by shrimp trawlers of Turtle Excluder Devices (TEDs) in 1990 could have presumably contributed to greater survival of small coastal sharks.

Alternatively, the relatively large number of small turtles in 2000 could be an anomaly and the other years may be more "natural." In other words, recruitment of loggerhead turtles from oceanic to neritic habitat may occur on a multiyear cycle. Regardless of the interpretation of the data, this apparent phenomenon warrants close scrutiny over the next several years.

Recaptures

Twenty-one previously tagged loggerhead turtles were recaptured during the project. Average time at-large was 2.01 years (minimum 16 days, maximum 8.95 years). Only two were recaptured within two months of release and most turtles were at-large for over a year (Figure 14). Ten of the twenty-one recaptured animals were both tagged and recaptured by this project. The remaining turtles were either tagged or recaptured by other researchers.

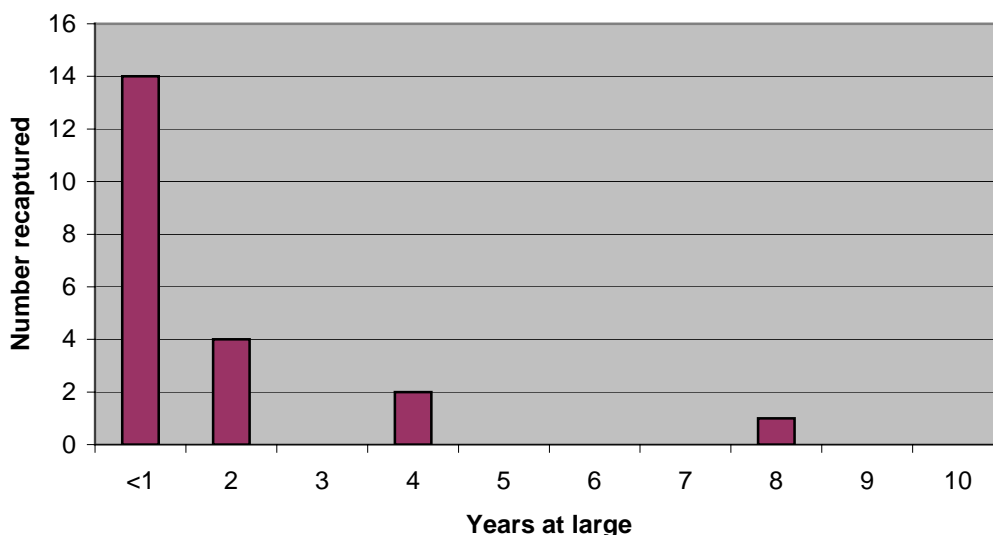


Figure 14. Time at-large before recapture of loggerhead turtles (N=21)

Eighteen of twenty-one recaptured animals were tagged and recaptured in our sampling area. The remaining three animals were tagged outside the sampling area by other researchers, and then subsequently captured in the sampling zone. An adult female tagged on Bald Head Island, NC in 1994 was recaptured in the northern sampling zone in 2003. One adult female and one juvenile loggerhead tagged at the St. Lucie Power plant in Florida were later captured by the project off Georgia.

In general, the point of recapture was not far from the initial point of tagging and release (Figure 15). Only three animals were collected more than 50 km from the site of release. Of these, one was the adult female tagged on Bald Head Island. A second adult female captured during the summer at the St. Lucie Power Plant was caught the next summer off Georgia. An immature turtle caught at the St. Lucie Power plant during the winter was caught several summers later off Georgia.

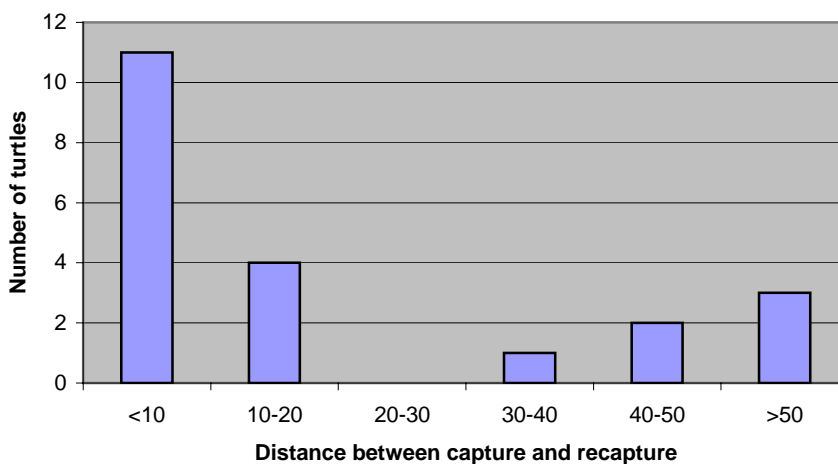


Figure 15. Distance between release and recapture sites in kilometers.

Neritic juvenile loggerheads included in our study seem to show fidelity to specific areas. All seventeen recaptured juvenile turtles were within 50 km of the point of release. Half of these were recaptured within 6 km of the point of release. This pattern of recapturing juvenile loggerhead turtles in the same general area was consistent regardless of the time between tagging and recapture (Figure 16). Comparison of the present study with the results of Van Dolah and Maier (1993) provides further support for the hypothesis of site fidelity in the feeding grounds in two ways. First, they sampled a very specific section of the Charleston channel thoroughly twice monthly for 16 months and showed a much higher project recapture rate (16.8%). The present project did not necessarily sample the same location each year, possibly contributing to the lower project recapture rate of 1.3%. Like the present study, 50% of the recaptures in Van Dolah and Maier (1993) occurred after the animals were absent in the samples from at least October through April, suggesting that they returned to the area after over-wintering in warmer waters. Anecdotal information from three adult females recaptured in the present project suggest a less consistent behavior with one adult female recaptured within 18 km and two over 200 km.

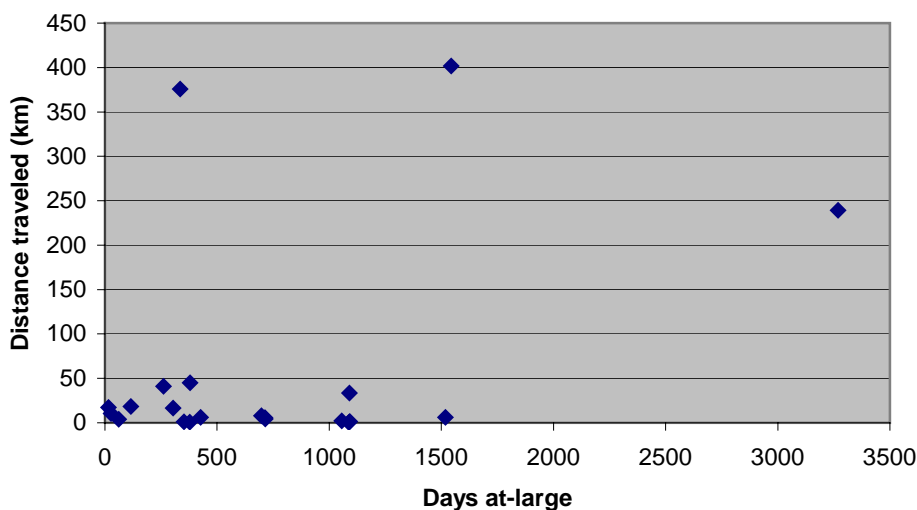


Figure 16. Relationship between time at-large and distance between location of release and recapture.

Genetics

Mitochondrial DNA haplotype data was used to evaluate the composition of loggerhead feeding aggregations and the management implications this genetic makeup has for nesting populations; particularly low-density nesting rookeries such as those found in the northern subpopulation. Since female sea turtles are strongly philopatric, maternally-inherited mtDNA haplotypes are similarly partitioned among nesting beaches, in some instances on a site-specific basis (Meylan *et al.*, 1990; Bowen *et al.*, 1992, 1993, 1994). The ability to differentiate individuals originating from genetically distinct nesting areas provides the basis for statistical estimates of an individual nesting area's contribution to offshore feeding aggregations using Mixed Stock Analyses (Grant *et al.*,

1980). Fortunately, Encalada et al. (1998) surveyed mtDNA variation of 249 individuals representing the major Atlantic Ocean and Mediterranean Sea loggerhead nesting populations, recovering six areas with significantly different haploype frequencies: Northeast Florida to North Carolina, USA (NEFL-NC), southern Florida, USA (SFL), Northwest Florida, USA (NWFL), Quintana Roo, Mexico (MEX), Bahia, Brazil (BRA) and Kiparissia Bay, Greece (GRE). We use these baseline genetic data to estimate the relative contribution of each area to juvenile feeding aggregations in coastal areas from North Carolina to northern Florida.

Whole blood (approximately 500 µl) was drawn from each individual, added to 9ml of lysis buffer (100mM Tris-HCL, 100mM EDTA, 10mM NaCl, 1.0% SDS; pH 8.0), and placed on ice. Total DNA was prepared using blood tissue samples using GeneReleaser following the manufacturer's (Bioventures) protocol. The mitochondrial DNA (mtDNA) control region was amplified via the Polymerase Chain Reaction (PCR) using primers CR-1 and CR-2 and amplification conditions described in Norman et al. (1994). Amplifications were performed in 50 µl reactions (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2.5 mM MgCl₂; 0.1% Tween 20; 5% DMSO; 200mM each dNTP, 10 pmol each primer and one unit of *Taq* DNA polymerase) in an MJ Thermal Cycler 6400 (MJ Research, Inc). Amplification products were purified by PEG precipitation and washed with 80% cold ethanol. A 1µl aliquot of purified amplification product was used as template in a Big Dye terminator cycle sequencing reaction (Applied Biosystems). All samples were sequenced in the forward direction using CR-1; some samples were also sequenced in the reverse direction with CR-2 to confirm haplotype designations. Sequencing reaction products were separated on an ABI 377 automated sequencer for 7 hrs at 28W constant power.

Partial (376 base pairs (bp)) mitochondrial control region sequences were obtained from 745 loggerhead turtles. Fifteen haplotypes were recovered (Table 3), seven of which correspond to previously published sequences (haplotypes A, B, C, G, H, I and J in Encalada et al. 1998). We retain the published haplotype designations for these seven. Eight previously unreported haplotypes were recovered and given designations on the basis of homology to published sequences followed by a unique number determined by order of observation; haplotypes A2 and A3 were most similar to the published A haplotype, while B2, B3, B4, B5, B6 and B7 were most similar to haplotype B (see Table 3).

Table 3. Mitochondrial DNA control region haplotypes observed in this study and nesting beach data from Encalada et al. (1998). See text for site abbreviations.

| Haplotype | Nesting Beach | | | | | | In-water samples | | | | Total of Years |
|-----------|---------------|-----|---------|-----|-----|-----|------------------|------|------|------|----------------|
| | NWFL | SFL | NEFL-NC | MEX | BRA | GRE | 2000 | 2001 | 2002 | 2003 | |
| A | 34 | 22 | 104 | | | | 117 | 98 | 124 | 58 | 397 |
| A2 | | | | | | | 7 | 4 | 6 | 2 | 19 |
| A3 | | | | | | | 1 | | 1 | | 2 |
| B | 4 | 24 | 1 | 11 | | 19 | 74 | 71 | 80 | 42 | 267 |
| B2 | | | | | | | 1 | 4 | | | 5 |
| B3 | | | | | | | 1 | 2 | 1 | 1 | 5 |
| B4 | | | | | | | 1 | | | | 1 |
| B5 | | | | | | | | 1 | | | 1 |
| B6 | | | | | | | | 1 | | | 1 |
| B7 | | | | | | | | | | | 1 |
| C | 2 | 2 | | 2 | | | 7 | 12 | 8 | 3 | 30 |
| D | | | | | 11 | | | | | | 0 |
| E | | 1 | | | | | | | | | 0 |
| F | | | | | | 2 | | | | | 0 |
| G | 2 | 1 | | | | | 4 | 2 | 1 | | 7 |
| H | | | | 1 | | | 1 | | | | 1 |
| I | | | | 1 | | | 2 | 1 | 2 | | 5 |
| J | | | | | | 5 | | 2 | 1 | | 3 |
| Total | | | | | | | 216 | 198 | 225 | 106 | 745 |

Two of twelve haplotypes (A, B) were present at a combined frequency of 89%, nearly identical to that observed among the six genetically distinct nesting areas described by Encalada et al. (1998). Haplotype C, found previously in six nesting individuals (two each in NWFL, SFL and MEX) was recovered from thirty individuals. Haplotypes G, H, I and J, found in a combined total of sixteen individuals, were also found in very low frequency in the nesting areas. Conversely, three haplotypes recorded in the nesting beach survey, two of which were rare (E, F) and one fixed in Brazil (D), were not recovered in this study.

To test for temporal variation between samples, the data were analyzed using the AMOVA (Excoffier *et al.*, 1992). The results indicate that all of the variation present is contained within, rather than between, temporal samples (Table 4). The lack of differentiation between years allowed for the pooling of these data for the subsequent analysis. The program SPAM (2000) estimates the contribution of each potential donor or baseline population (six genetically distinct nesting areas for loggerhead turtles) to a mixed stock of individuals (offshore feeding aggregations). Implementing this approach, Florida subpopulations (NWFL and SFL) had a disproportionate estimated contribution to the feeding aggregation (66%); estimated contribution of the nesting area north of St. Augustine, FL (NEFL-NC) was 19% (Table 5). All other source populations were estimated to be infrequent contributors. These estimates were robust to the exclusion of haplotypes not present in the potential source populations.

Table 4. AMOVA table for evaluating differences between temporal samples.

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation |
|---------------------|------------|----------------|---------------------|-------------------------|
| Among populations | 3 | 0.436 | -0.00081 V_a | -0.28 |
| Within populations | 741 | 217.499 | 0.29352 V_b | 100.28 |
| Total | 744 | 217.936 | 0.29271 | |
| Fixation Index | F_{ST} : | -0.00278 | | |

Table 5. Estimated contributions of nesting regions to loggerhead samples. Standard errors are in parentheses.

| Sample Year | Nesting Beach | | | | | | |
|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | NWFL | SFL | NEFL-NC | MEX | BRA | GRE | UNK |
| 2000 | 0.18 (0.17) | 0.46 (0.20) | 0.19 (0.15) | 0.05 (0.04) | 0.00 (0.00) | 0.06 (0.09) | 0.05 (0.09) |
| 2001 | 0.17 (0.17) | 0.53 (0.21) | 0.14 (0.13) | 0.06 (0.05) | 0.00 (0.00) | 0.04 (0.08) | 0.06 (0.08) |
| 2002 | 0.11 (0.15) | 0.49 (0.23) | 0.25 (0.16) | 0.04 (0.04) | 0.00 (0.00) | 0.07 (0.11) | 0.04 (0.11) |
| 2003 | 0.08 (0.15) | 0.54 (0.29) | 0.25 (0.17) | 0.04 (0.02) | 0.00 (0.00) | 0.10 (0.14) | 0.03 (0.14) |
| Pooled | 0.15 (0.16) | 0.51 (0.21) | 0.19 (0.14) | 0.04 (0.03) | 0.00 (0.00) | 0.06 (0.09) | 0.05 (0.09) |

Available data point to segregation by genetically distinct nesting areas among feeding aggregations in the Atlantic. Epifaunal characteristics and heavy metal accumulations in juvenile and adult loggerhead turtles suggest some segregation among offshore aggregations according to natal origin, specifically between the NEFL-NC and NWFL/SFL nesting areas (Stoneburner *et al.*, 1980; Caine, 1986). Similarly, Meylan *et al.* (1983) indicated that loggerheads from Florida nesting beaches feed preferentially in the Caribbean or Gulf of Mexico, while loggerheads from the NEFL-NC nesting beaches have foraging areas from mid Florida to New Jersey (S.R. Hopkins-Murphy, pers. comm.). Therefore, juvenile turtles in the NEFL-NC foraging area should disproportionately represent nearby nesting beaches, a hypothesis that has important implications for the management of loggerhead turtles. Impacts on the NEFL-NC offshore aggregation would negatively threaten future reproductive output of NEFL-NC beaches and thus the persistence of this genetically distinct nesting area.

The majority of offshore turtles in the NEFL-NC feeding area were derived from the Florida nesting assemblage (66%). However, a significant proportion of offshore individuals (19 %) were from the geographically proximate NEFL-NC nesting area. This NEFL-NC contribution is significant for several reasons. The NEFL-NC nesting area

contains only nine percent of the loggerhead nesting activity along the Atlantic Coast (NMFS/USFWS, 1991), but mixed stock analysis assigned greater than 19% of the feeding aggregation to this area. This observation is consistent with mixing of individuals from various nesting assemblages in offshore areas and concentration of juveniles from NEFL-NC beaches in offshore feeding areas – the NEFL-NC nesting area is contributing disproportionately to nearby offshore feeding aggregations. Therefore, mortality to juveniles in the NEFL-NC feeding grounds will have a disproportionate effect on reproductive viability of the NEFL-NC nesting area. Although the estimated frequency of NEFL-NC individuals in the offshore aggregation was low (19%), this proportion might represent the majority of the reproductive output of the NEFL-NC nesting area.

The offshore distribution of loggerhead turtle haplotypes in the NEFL-NC area has profound conservation implications. Juvenile turtles hatched from this area appear to annually return to nearby offshore feeding areas during the warm weather seasons. Thus, juvenile mortality in the offshore assemblage could significantly compromise the reproductive viability of the NEFL-NC populations. Furthermore, turtle mortalities occurring on these feeding areas could compromise other populations as well. The majority (66%) of the turtles represented Florida nesting populations and small percentages (approximately 5% each) represented infrequent, but potentially important, contributions from Mexico and the Mediterranean. Also, 5% of the turtles possess haplotypes not encountered in any of the nesting populations. These haplotypes might represent another unidentified, but genetically identifiable, nesting population for which this is a critical feeding area.

The robustness of an MSA is improved by performing a comprehensive survey, with large sample sizes, of potential stock populations that are highly distinct. Widespread marine species with widely distributed nesting areas are unlikely to conform to these criteria. While these six nesting regions represent the overwhelming majority of nesting in the Atlantic and Mediterranean, and therefore are the most likely candidates for source populations, characterization of smaller populations along the western coast of Africa and elsewhere in the Mediterranean is needed. Stock populations that exhibit fixed differences are evidently more precise demographic estimators of mixed populations. This remains a variable beyond control, however, large sample sizes allow for the discrimination of unique haplotypes, which are uninformative, from haplotypes which occur in low frequencies and are informative thus increasing our ability to robustly differentiate populations. Xu et al. (1994) demonstrated that the effect of increasing the sample size of mixed populations is negligible relative to the effect of increasing the sample size of the baseline populations. Therefore, increasing the baseline sample sizes is necessary to more confidently describe the mixed populations and generate the precise values needed to make informed policy decisions.

While large standard errors associated with the present study necessitate cautious interpretation of the data, it is clear that for juvenile sea turtles, such as sampled for this study, represent what should now be a primary focus of conservation efforts. No amount of protection afforded to nesting beaches can be successful without an adequate supply of sexually mature individuals to continually supply nests. Protection of juvenile loggerheads while on these warm-season feeding grounds is important to the recovery of the small and declining nesting populations in NEFL-NC as well as other areas.

Factors Affecting Catch Rates

Turtle catch rates in an assessment study of this type may be affected by a number of factors unrelated to turtle abundance. Because of the scale of this project and the variety of conditions encountered during sampling, we have a somewhat unique opportunity to examine the effects of environmental and other factors upon turtle catch rates.

Eight environmental and project-related variables measured with each tow were assessed for their affect on catch of loggerhead turtles. Analysis proceeded in the same manner for each variable. First, presence or absence of a turtle in each tow was established. The measures for the variable being analyzed were then categorized over the range of observed values to produce approximately five categories. Finally, Pearson chi-square analysis was used to determine whether presence of a turtle in a tow was related to the levels of the categories.

Results and Discussion

Tow Speed

Sea turtles, when encountering trawls, are known to swim ahead the trawl in an effort to evade the trawl. As they tire and lose forward speed, the trawl eventually overtakes the turtle, thus capturing it (Ogren *et al.*, undated). Therefore, towing speed is likely to be an important variable affecting capture of sea turtles. It may be assumed that faster towing speeds may increase catch rates of sea turtles, provided the gear continues to fish as designed at higher speeds.

Onboard GPS units determined tow speeds of the fishery-independent vessels of this study. The tow speed of vessels averaged 2.75 nautical miles per hour, with 90% of the tows conducted between 2.5 and 3.0 nautical miles per hour. Presence of turtles was significantly different among tows at differing speeds ($P=0.006$). For our nets, trawl speeds below 2.5 nautical miles per hour proved considerably less effective in catching turtles (Figure 17).

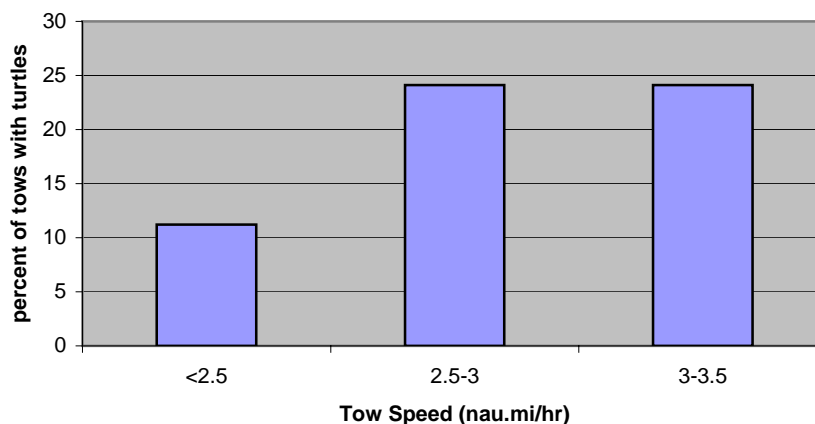


Figure 17. Relationship of tow speed and presence of turtles

Wave Height

Sampling was conducted in seas ranging from 0 to 1.52 m (5 ft) as assessed by the vessel captain and data recorder. It should be noted that while the range of wave heights for which samples were collected is considerable, 95% of the tows were conducted in seas less than 0.61 m (2 ft) in height. Given the relatively calm conditions and large vessels used in the project, sea condition probably had little effect on vessel maneuverability or towing ability. For analysis, stations and catch at each station were partitioned into five 0.305 m (1-ft) categories for sea height. Sea state did not significantly affect whether a turtle was caught in a tow ($P=0.373$).

Water Temperature

Water temperatures observed during the study ranged from 22.2°C to 31.3°C. The majority of tows (74.9%) were conducted at temperatures between 26°C and 29°C. For analysis, catch data were partitioned by stations with temperatures between 26 and 29°C in 1° increments and for those locations equal to or above 29°C, and below 26°C. Initial chi-square analysis indicated a significant difference in catch rates among the five data groups. Catch rates for temperatures below 26°C were significantly lower than those for other all temperature classes (Figure 18). Subsequently, all tows above 26°C were lumped and compared (chi-square test) to those below 26°C; catch rates at the cooler temperatures were significantly less than those of the combined higher temperatures ($P=0.0018$).

Sea turtles are known to be less active in cooler temperature and appear to actively avoid them. Although the range of temperatures observed in this study appeared to be well within the normal range of preferred temperatures, it appears that there may be some avoidance of temperatures below 26°C or preference for temperatures above 26°C.

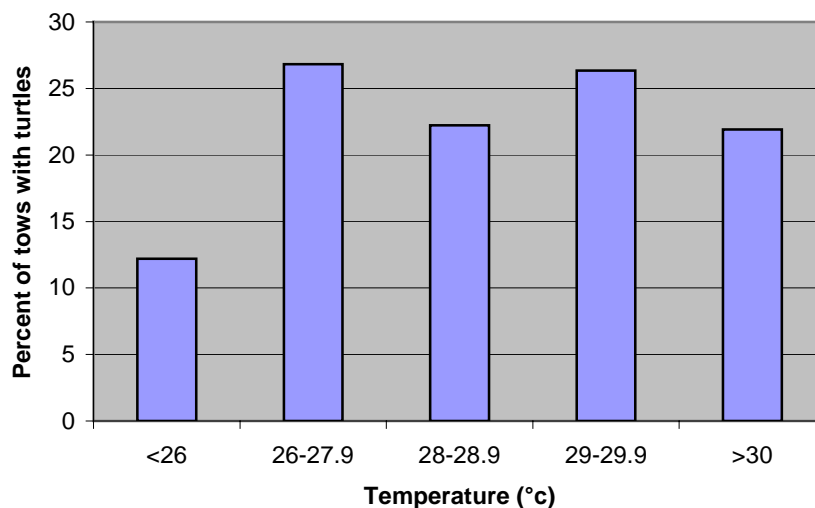


Figure 18. Affect of temperature on presence of turtles.

Latitude

The Abundance chapter of this document discusses in detail the affect of region on CPUE. The analysis provided here should be considered a supplement to that more thorough analysis.

For this analysis, latitude determined from GPS units for the start of each tow was first converted to decimal degrees and tows were then grouped into 0.5-degree increments from 30 to 33.5 degrees. Chi-square analysis detected a significant difference in the presence of turtles in tows among the resulting seven groups ($P < 0.001$). Loggerhead turtles were more commonly caught in trawls at lower latitudes of our sampling area (Figure 19). These data support the conclusion from the Abundance chapter of this paper that regional differences existed in catch, with turtles being caught more frequently in the southern portion of our sampling area.

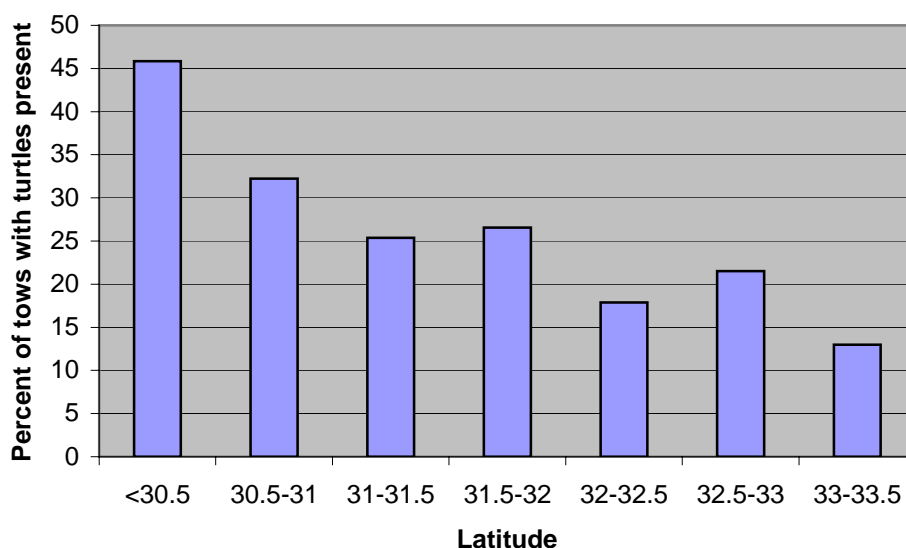


Figure 19. Relationship of latitude and presence of turtles.

Depth

Depth at which a tow was conducted did not significantly impact presence of turtles in the catch, but tows conducted in the 20-30 ft depth range had a higher percentage of tows with turtles than all other depths ($P = 0.002$, Figure 20).

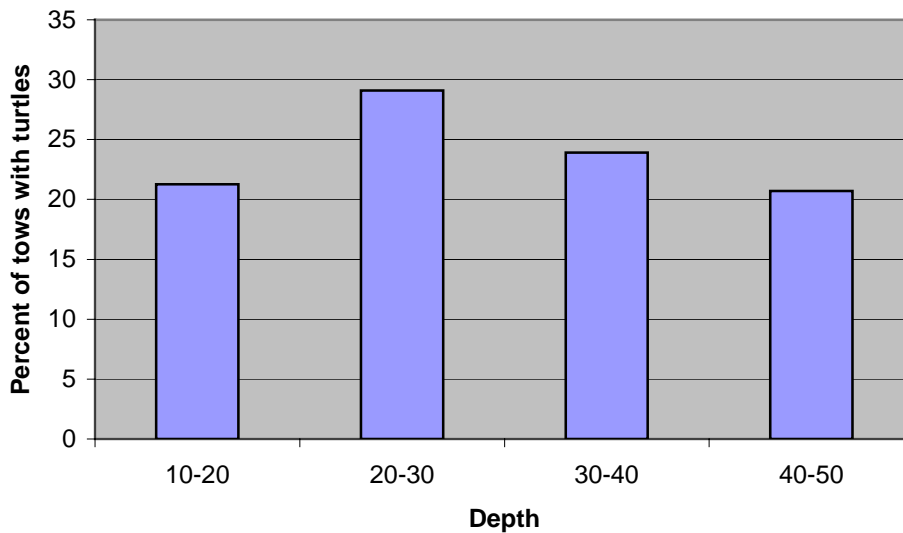


Figure 20. Relationship of depth and presence of turtles.

Cloud Cover

Cloud cover was estimated as percent of sky covered. For chi-square analysis, catch data for the tows were placed into one of five groups each corresponding to 20% intervals of cloud cover. Statistical difference in presence of turtles in the catch was not detected among cloud cover groups ($P=0.9515$).

Wind Speed

Presence of turtles in tows was compared over the range of wind speeds encountered during the sampling. Tows were grouped by wind speed into 5-mph groups from 0-20 mph; tows with wind speeds greater than 20 mph comprised the final group (Figure 21). Chi-square analysis indicated that wind speed had a significant affect on presence of turtles in the trawls ($P=0.0173$) with fewer turtles being taken at the higher wind speeds. It is unknown if this condition resulted in less efficiency of the net (i.e., the net was off the bottom) or if the turtles retreated to deeper, calmer waters in areas offshore of our sampling zone. This finding suggests that turtle assessments using similar gear should probably suspend sampling operations when winds exceed 15 mph to minimize variance in the data.

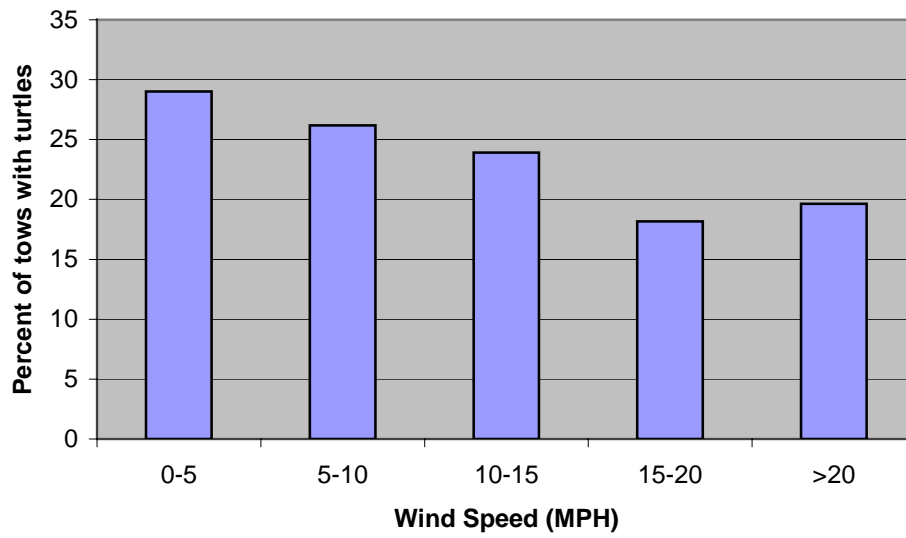


Figure 21. Relationship between wind speed and presence of turtles.

Morphometrics

Morphometric measurements, along with body weight, age estimates, and reproductive data, provide critical biological information for population assessments and management of natural resources. In fisheries management, age-specific growth rates and age at maturity are routinely used in stock assessment models (Hilborn and Waters, 1992); however, the best methods for determining age and reproductive biology often require sacrificing animals or conducting highly invasive surgical procedures, procedures which are not always feasible. In the absence of age and reproductive data, morphometric relationships and body weight provide useful information for population and health assessments. For example, the relationship between body length and body weight may reflect fluctuations in the uptake and allocation of energy (Pérez-Castaneda and Defeo, 2002); thus, providing a useful measure for comparing species-specific growth rates throughout a geographical distribution range. Similarly, relationships between two or more morphometric characters may provide a simplistic means for assessing the origin or sex of individuals without complex and expensive laboratory-based assessments.

Sea turtles, which are not sexually dimorphic until perhaps 25 years old, and cannot be collected or sacrificed due to their federally protected status, represent ideal animals for use of morphometric measurements to assess subpopulation or other trends. In the Western Atlantic Ocean, three species of hard-shelled sea turtles (loggerheads, Kemp's ridley, and green sea turtle) regularly appear along the Eastern Seaboard of the United States during the summer months to nest and/or forage. Seasonal occurrence and distribution of these sea turtles has been documented using shore and aerial-based techniques. Studies of sea turtles along the Atlantic coast have provided much information, particularly the adult nesting females (Lutcavage and Musick, 1985; Hopkins-Murphy *et al.*, 1999, Parham and Zug, 1997 and Meylan *et al.*, 1995). However, comparatively less information exists for other segments of the population, such as adult males and juveniles. Much of the data collected for adult and juvenile sea turtles comes from stranded sea turtles. Because stranded sea turtles are often dead and necrotic or emaciated, morphometric measurement data from these animals may be compromised and not indicative of the larger population.

Capture of sea turtles by trawling provides an opportunity to sample free-swimming sea turtles, particularly adult males and juvenile males and females, not readily accessible using traditional sampling approaches (i.e., nesting and aerial surveys). Here we present morphometric relationships for live, non-stranded sea turtles caught by fishery-independent means along the southeastern coast in summer 2000-2003.

Methods

Measurement precision (Bolten, 1999) was evaluated for a subset of loggerheads in the first year of the study. Duplicate measurements were recorded for most sea turtles caught in the northern and southern regions of the project study area during the first two weeks in July 2000. Following initial measurements, these turtles were measured a second time using a partially blind design.

Correlation analyses (Microsoft Excel®) were used to compare straight-line and curved measurements for minimum carapace length, carapace length (notch-tip), and

carapace width for loggerheads and Kemp's ridleys; sample size for green sea turtles was too small ($n=7$) to meaningfully compute relationships. Correlation analyses (Microsoft Excel®) were also used to evaluate morphometric relationships between straight-line carapace length (SCLmin) and six other measurements (body weight, straight-line carapace width (SCW), body depth (BD), curved plastron width (PW), head width (HW), and tail length (plastron to tip of tail)).

Results

Eight hundred eighty-nine sea turtles (827 loggerheads, 55 Kemp's ridleys, and 7 green sea turtles) were collected in 2000-2003 (Figure 22). Ninety-two percent of loggerheads were larger (SCLmin) than the largest Kemp's ridleys; however, considerable overlap in size was observed between Kemp's ridleys and green sea turtles, such that all green sea turtles were larger than the smallest Kemp's ridleys (Figure 22). Mean carapace lengths (SCLmin) of loggerheads, Kemp's ridleys, and greens were 67.5 cm (range = 44.8 – 103.5 cm), 45.4 cm (range = 26.7 – 62.1 cm), and 29.5 cm (range = 27.6 – 30.6 cm), respectively.

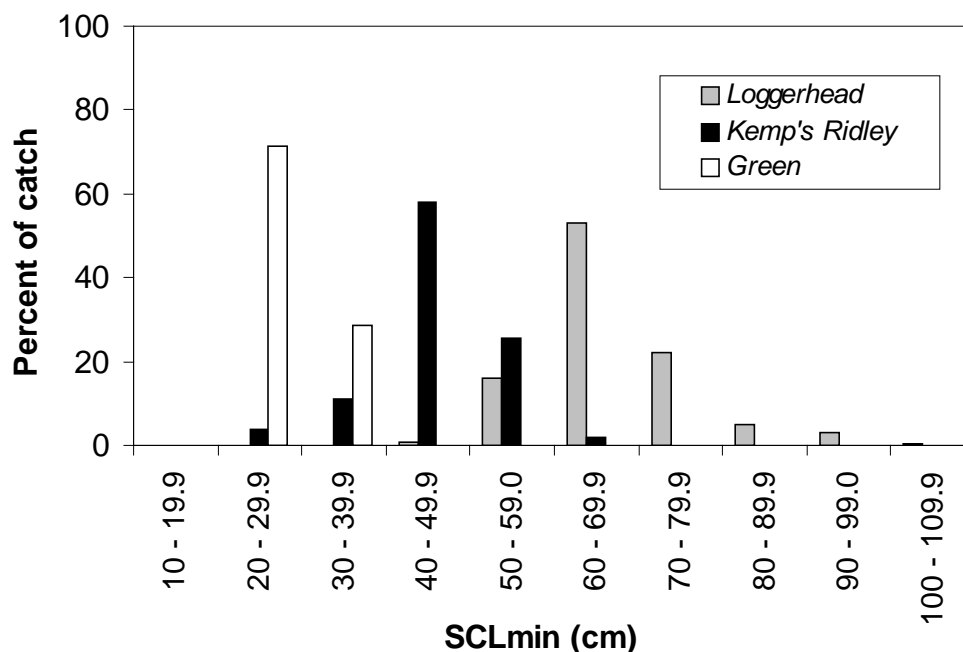


Figure 22. Size-frequency distribution of sea turtles collected by fishery-independent bottom trawling in South Carolina to northern Florida waters, summer 2000-2003. Carapace length (SCLmin) data were not available for five *Caretta caretta*.

Measurement error was most pronounced for measurements of mobile body parts (Table 6). Tail length and head width measurements were the most variable, with maximum percent differences of >40%. Body depth precision was also variable; however, sample size for precision estimates of this parameter was approximately half that used to estimate precision for other parameters. Conversely, measurements were highly precise for both straight and curved measurements of carapace length and width, with mean differences in precision typically less than 1% for these six measurements.

Table 6. Measurement precision, determined via a second set of measurements collected for a sub-set of *Caretta caretta* in July 2000, using a partially blind design.

| Parameter | n | Mean % Diff | Min % Diff | Max % Diff |
|------------------|----------|--------------------|-------------------|-------------------|
| SCLmin (cm) | 17 | 0.34 | 0 | 0.76 |
| SCLnt (cm) | 17 | 0.37 | 0 | 1.44 |
| SCW (cm) | 17 | 1.07 | 0 | 3.21 |
| CCLmin (cm) | 16 | 0.64 | 0 | 2.7 |
| CCLnt (cm) | 16 | 0.64 | 0 | 1.96 |
| CCW (cm) | 16 | 0.66 | 0 | 1.83 |
| HW (cm) | 16 | 5.56 | 0 | 43.09 |
| TLct (cm) | 16 | 9.99 | 0 | 50 |
| TLpt (cm) | 15 | 3.81 | 0 | 23.71 |
| BD (cm) | 7 | 5.84 | 0 | 25 |

Straight-line and curved measurements for minimum carapace length were highly correlated for loggerheads (n=821, $R^2=0.97$, Table 7) and Kemp's ridleys (n=55, $R^2=0.99$, Table 7). Curved minimum carapace length exceeded straight-line minimum carapace length by 8% (range = 1.2% to 16%) on average for loggerheads, compared to an average 6% difference (range = 3% to 10%) between curved and straight-line measurements for minimum carapace length among Kemp's ridleys.

Table 7. Relationships between straight-line and curved carapace length (CLmin, CLnt) and carapace width (CW) for *C. caretta* and *L. kempii* sea turtles.

| Species | N | R2 | Equation |
|----------------|----------|-----------|-------------------------------------|
| Loggerhead | 821 | 0.98 | $CCLmin = 1.0460 (SCLmin) + 2.7131$ |
| Kemp's ridley | 55 | 0.99 | $CCLmin = 1.0345 (SCLmin) + 1.2139$ |
| Loggerhead | 825 | 0.98 | $CCLnt = 1.0369 (SCLnt) + 3.1655$ |
| Kemp's ridley | 55 | 0.99 | $CCLnt = 1.0370 (SCLnt) + 0.8971$ |
| Loggerhead | 814 | 0.89 | $CCW = 1.2682 (SCW) - 0.0785$ |
| Kemp's ridley | 55 | 0.98 | $CCW = 1.1107 (SCW) + 1.1126$ |

Straight-line and curved measurements for notch-tip carapace length were highly correlated for loggerheads (n=825, $R^2=0.98$, Table 7) and Kemp's ridleys (n=55, $R^2=0.99$, Table 7). Curved minimum carapace length exceeded straight-line minimum carapace length by 8% (range = <1% to 16%) on average for loggerheads, compared to

an average 5% difference (range = 2% to 9%) between curved and straight-line measurements for notch-tip carapace length among Kemp's ridleys.

Straight-line and curved measurements for carapace width were moderately correlated for loggerheads ($n=814$, $R^2=0.89$, Table 7), but highly correlated for Kemp's ridleys ($n=55$, $R^2=0.96$, Table 7). Curved minimum carapace width exceeded straight-line minimum carapace width by 21% (range = 3% to 30%) on average for loggerheads, compared to an average 12% difference (range = 4% to 17%) between curved and straight-line measurements for carapace width among Kemp's ridleys.

Moderate ($R^2 = 0.80$ to 0.88) linear relationships were observed between minimum straight-line carapace length and (1) straight-line carapace width (cm), (2) head width (cm), (3) body depth and (4) body weight (kg) for loggerheads (Table 8). A moderate quadratic ($R^2=0.91$) relationship between straight-line carapace length and body weight (kg) was observed for loggerheads (Table 8; Figure 23). With the exception of body weight, R^2 values for the morphometric relationships for Kemp's ridleys were higher than R^2 values (by 0.04 to 0.09) for the same relationships for loggerheads, although the respective sample size was about 1/16th that of loggerheads.

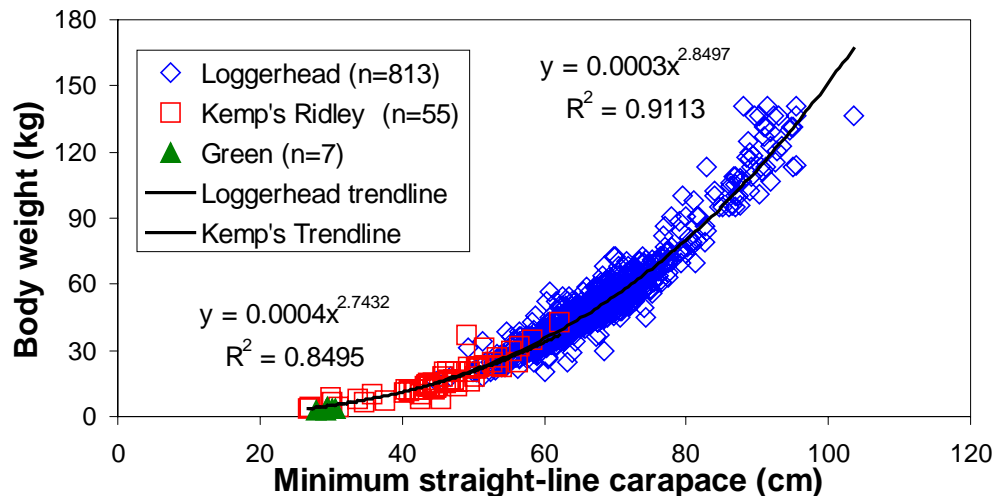


Figure 23. Relationship between carapace length and body weight for sea turtle species in coastal waters of the southeastern U.S., 2000-2003.

Poor linear relationships ($R^2 < 0.70$) were observed for straight-line carapace length and curved tail length (plastron-tip, cm) for both loggerheads and Kemp's ridleys (Table 8), even after the removal of apparently mature male loggerheads. Given the high precision error noted for this measurement, slight differences in the correlation equations for minimum straight-line carapace versus tail length for males and females were not considered informative; however, proportionate tail length (assuming consistent precision error for both males and females) was useful for distinguishing between male and female loggerheads >85 cm SCLmin (2000-2002 data, Figure 24). Tail length only exceeded 30% of straight-line minimum carapace length in 1 of 372 females (0.3%), compared to tail lengths $> 30\%$ of straight-line minimum carapace length in 18 of 162 males (11.1%). Furthermore, only males were observed with tail lengths $>40\%$ of straight-line minimum carapace length for individuals > 85 cm SCLmin (Figure 24).

Table 8. Summary of correlation analyses for morphometric measurements.

| Loggerhead | N | R ² | Equation |
|---------------------------|------|----------------|------------------------|
| SCLmin vs. Body weight | 813 | 0.91 | $y = 0.0004x^{2.8497}$ |
| SCLmin vs. Carapace width | 813 | 0.88 | $y = 0.6337x + 12.331$ |
| SCLmin vs. Head width | 821 | 0.88 | $y = 0.2185x - 0.5728$ |
| SCLmin vs. Body depth | 702 | 0.8 | $y = 0.3520x + 4.2478$ |
| SCLmin vs. Tail length | 795* | 0.62 | $y = 0.3040x - 5.8653$ |

*Loggerhead turtles with tail lengths > 30% of SCLmin were excluded from correlation.

| Kemp's Ridley | N | R ² | Equation |
|---------------------------|----|----------------|------------------------|
| SCLmin vs. Body weight | 55 | 0.85 | $y = 0.0002x^{2.7432}$ |
| SCLmin vs. Carapace width | 55 | 0.97 | $y = 1.0344x - 1.7515$ |
| SCLmin vs. Head width | 55 | 0.92 | $y = 0.1778x + 1.9746$ |
| SCLmin vs. Body depth | 48 | 0.84 | $y = 0.3490x + 1.6292$ |
| SCLmin vs. Tail length | 55 | 0.67 | $y = 0.2726x - 2.6259$ |

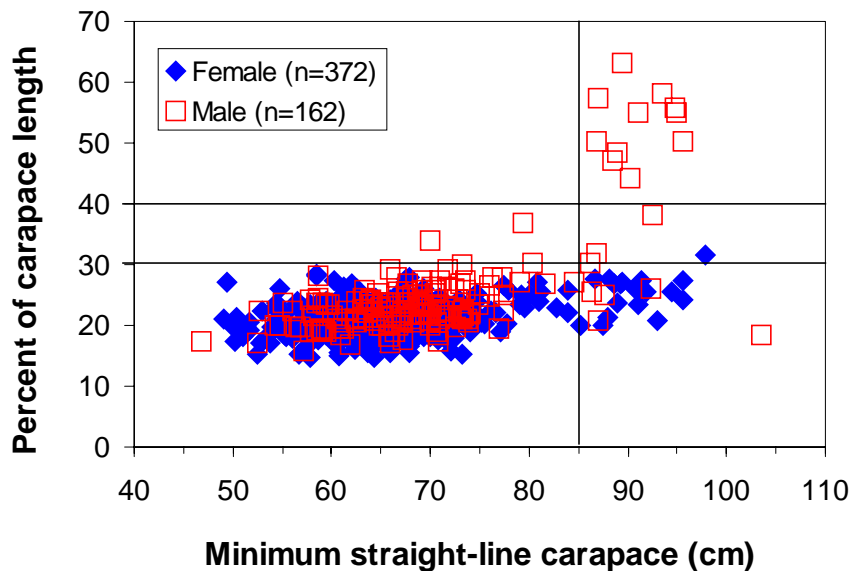


Figure 24. Tail length as an indication of sexual dimorphism in *C. caretta*.

Discussion

Loggerhead sea turtles comprised 93% of sea turtles collected in summers 2000-2004. Seventy percent of loggerheads measured 60-74.9cm SCLmin, with remaining turtles nearly evenly distributed among < 60cm or ≥ 75cm SCLmin groupings. Relative

species abundance and size distribution for loggerheads collected in this study was similar to that observed in previous trawling studies in coastal South Carolina waters (Ulrich, 1978,1980; Van Dolah and Maier, 1993). Along the eastern seaboard, similar relative species abundance and size distribution of loggerheads are reported from trawl-caught turtles in central Florida (Schmid, 1995) and from stranding data as far north along the U.S. eastern seaboard as Virginia Lutcavage and Musick, 1985). Proportionately fewer and smaller loggerheads are reported from (winter) stranding data in New York waters (Morreale *et al.*, 1992).

Measurement precision was strongly influenced by the mobility or pliability of the anatomical part being measured; however, movements of animals being measured occasionally rendered all measurements difficult. Measurement error was most pronounced for head width, body depth, and tail length measurements. Similar trends in measurement error were observed for non-explicit measurements (i.e., non-carapace measurements) among stranded turtles measured in Virginia waters (Coles, 1999). Carapace length and width were highly precise for both straight and curved measurements. Biotic fouling occasionally compromised the accuracy of curved carapace measurements and physical damage to the carapace occasionally affected the accuracy of both straight-line and curved carapace measurements. Heavy biotic fouling or physical damage to the carapace was observed in approximately 15% of loggerheads in this study (See section on Turtle Health).

Curved measurements of carapace length (CCLmin, CCLnt) and carapace width exceeded straight-line measurements for the same dimension for both loggerheads and Kemp's ridleys. Strong linear relationships were detected between straight-line and curved measurements for carapace length and width for both species. Slope for the conversion equation between straight-line and curved carapace lengths for loggerheads (1.04) was similar to slopes reported for the same conversion for stranded loggerheads in the Gulf of Mexico and Southeast Atlantic waters (1.05, n=932, Teas, 1993) and for nesting loggerheads at the Kennedy Space Center in Florida (1.02, n=366, Frazer and Ehrhart, 1983); however, y-intercept values were nearly double that reported by Teas (1993) and half the value reported by Frazer and Ehrhart (1983).

Discrepancies in these equations are likely a result of size and location of animals collected. In the current study, predominantly immature loggerheads were collected from a moderate geographical range. The data presented by Teas (1993) consist of many different-sized animals from a very large geographical area, and the Frazer and Ehrhart (1983) data are derived from many similar-sized animals (i.e., nesting females) from a very localized area. Morphological variation among adult female loggerheads has been reported from nesting beaches within the southeastern U.S. (Stoneburner, 1980) and from nesting beaches in the southeastern U.S. and other ocean basins (Tiwari and Bjorndal, 2000). It is unclear whether the differences between findings from the current study and previous works represent differences in carapace curvature, which has been shown to decrease with increasing carapace length for stranded, predominantly juvenile loggerheads from Chesapeake Bay and coastal VA waters, (Coles 1999).

Carapace curvature can increase the amount of lift and drag exerted on the carapace, such that greater lift is generated from highly domed carapaces, which in turn may be less energy efficient for long-distance migrations (Wyneken, 1997). Angle measurements necessary for determination of carapace curvature were not determined in

this study; however, surrogate variables for carapace curvature (percent difference between straight-line and curved measurements for (1) minimum carapace length and (2) carapace width) were examined. Scatter plot analysis of each of these variables with respect to minimum straight-line carapace length did not reveal any obvious relationships with respect to body size, temporal, spatial, or other partitioning. The inability to distinguish small-scale geographic variation in origin among individual turtles with the same genetic haplotype is likely responsible for the inability to match potential phenotypes with genotypes in this study.

Length-weight relationships are frequently determined for fishes both because these parameters are easy to measure and because the relationship between length and weight provide insight into growth rates and body condition. Although body mass is the most biologically significant measure because physiological and thermo-regulatory parameters scale to mass (Bjorndal and Bolten, 1988a,b), body weight is rarely recorded for sea turtles primarily because of the impracticality of weighing turtles on beaches or in small boats. Furthermore, reproductive condition, nutrition, and ingestion/eggestion can result in daily and seasonal variability in length-weight relationships (Dunham, 1978; Pough, 1980; Balazs, 1982; Bjorndal and Bolten, 1988a).

Length-weight relationships were reported in this study; however, due to the similarity between relationships presented here and previous studies, length-weight relationships were not useful for distinguishing loggerheads among geographic regions within the geographic range of this study. Coles (1999) reported almost the same quadratic relationship between body weight and curved carapace length (CCLmin) for loggerheads ($y=0.0004x^{2.7108}$, $R^2=0.91$, $n=415$) as reported in this study; however, an exponential relationship was observed for Kemp's ridleys ($y=9e-5x^{3.0786}$, $R^2=0.95$, $n=79$) stranded in Virginia waters. The occurrence of exponential versus quadratic relationships in the Virginia study may have resulted from the use of a surrogate weight value mathematically determined from carapace curvature, rather than use of the actual weight of each animal. Schmid (1995) did not report on length-weight relationships for loggerheads at Cape Canaveral, Florida; however, curvilinear relationships were reported for Kemp's ridleys ($n=88$) in Cape Canaveral, Florida, and also from west-central Florida ($n=225$). Parameters were transformed to generate linear equations for Kemp's ridleys along the east ($\log wt = -8.2837 + 2.844 (\log SCLmin)$; $r=0.97$; Schmid 1995) and west coasts ($\ln wt = -8.1570 + 2.8128 (\ln SCLmin)$; $r=0.98$; Schmid 1999).

With the exception of tail length, morphometric relationships were not useful in distinguishing individual loggerheads with respect to sex or genetic origin. In this study, approximately twice as many females were collected as males and a mixture of genetic haplotypes were observed, consistent with gender patterns for this species along the eastern seaboard of the US (Wibbles *et al.*, 1991) and for genetic analyses of trawl-caught individuals in the vicinity of Charleston Harbor, SC (Sears *et al.*, 1995). The inability to use morphological characters to distinguish genetic differences in sea turtles was also observed by Coles (1999) for a presumably genetically- and gender-mixed population of juvenile and adult sea turtles in Virginia.

Estimated Total Number of Commercial Shrimp Trawler/Loggerhead Sea Turtle Interactions off the South Carolina Coast During June and July 2001-2003

Overall loggerhead sea turtle catch rates recorded in the fishery-independent portion of this study were used to develop an estimate of the number of total interactions of shrimp boats with loggerhead sea turtles during the time period of this study. Simply put, the average catch-per-unit-effort by the fishery-independent trawlers was multiplied by an estimate of total shrimp trawler fishing effort to yield the estimated total number of interactions. Interaction is defined here as the likely catch or passage of a sea turtle through a shrimp trawl and shrimp turtle excluder device (TED). An analysis of this type could conceivably provide some insights into the function and “success rates” of currently employed TEDs.

Assumptions

An analysis of this type is inherently rife with assumptions. Estimates of fishing effort in the shrimp fishery are notoriously difficult to calculate. Management agencies rely upon reports from fishermen and there appears to be a great deal of variability in the precision that fishermen report fishing effort. The following assumptions were made regarding the analysis:

- The average number of nets per boat equals 2.91. This number was based upon work done in South Carolina by Henry, *et al.* (2001) and conversations with DNR economist Ray Rhodes. We assumed that vessels between 31 and 60 feet fished two nets and vessels greater than 60 feet used four nets. A mean was developed by summing all values for the two size classes.
- The average commercial net size was 40 feet. The larger trawlers typically tow four nets and our observations suggest 40 feet may be a good average size. No reliable, hard data on net size exist at this time. Smaller vessels that usually fish only two nets will have nets from 40 to 70 feet. Overall, we are probably underestimating average net size.
- The fishery-independent data were representative of commercial trawlers. An obvious difference in gear used in the fishery-independent phase and that used by commercial trawlers was the mesh size of the nets. The research trawlers used 8-in. stretch mesh nets compared to the 1 7/8-in. or 2-in. stretch mesh typically used in the shrimp trawling fishery. The 8-in. mesh could have resulted in proportionally wider spreading nets, thus disallowing the assumption of a linear relationship between catch rate with regard to net size, and the larger-mesh nets probably resulted in slightly higher towing speeds. Tow speeds with the turtle nets averaged 2.75 knots (std dev = 0.21), which is slightly faster than typical for commercial trawlers that trawl at about 1.8-2.2 knots. However, turtle catch rates in the fishery-dependent phase of this study were generally higher than catches in the fishery-independent phase.
- The areas sampled by the fishery-independent trawling using a stratified random sampling design were comparable in terms of turtle abundance on the “shrimp fishing grounds.” Catch rates in the fishery-independent phase of this study were

remarkably consistent from year to year giving us some assurance that our methodology was sound in terms of repeatability. The random sampling nature of this study resulted in a large portion, and maybe a majority of the samples, being taken in areas not considered traditional shrimping areas. However, all these areas were open to the commercial shrimp trawlers. We have not closely compared our turtle CPUEs for areas fished and not fished by shrimp trawlers, but based upon experience and intuition, we suggest that loggerheads, at least juveniles, are probably more abundant in areas regularly fished by shrimp trawlers. The assumption is that the turtles are feeding primarily near inlets where shrimp are typically more abundant. We believe that our fishery-independent CPUEs may actually be less than the actual interaction rates with commercial shrimp trawlers.

Calculations

A new catch-effort system was begun in South Carolina in September 2003. Accurate daily effort data were not recorded prior to that date, necessitating assumptions regarding hours fished per trip. However, estimated numbers of trips for each month was available with a trip being defined as the time period (days) between unloadings. We assumed that an average trip had 12 hours of actual gear in the water. Trawlers may unload after single day trips, but others often go several days between unloadings.

Table 9. Experimental fishing CPUE shown as turtles/30min tow/60-ft net. Parentheses indicate numbers of tows.

| | June | July |
|-------------|----------------|----------------|
| 2001 | 0.107 (346) | 0.144 (132) |
| 2002 | 0.092 (498) | 0.096 (115) |
| 2003 | 0.138 (522) | 0.083 (72) |

Assuming a linear relationship between turtle catch rate and net size, the average catch per tow for the 60-ft net (30-min. tow) from the research trawler was converted to that of a 40-ft net (estimated size of SC nets). To determine the number of turtles per 40 ft net/hour, this adjusted value was doubled. Values were then multiplied by an estimated average number of nets per boat of 2.91 (Henry *et al.*, 2001) to provide average number of turtle interactions per boat per hour. This value was multiplied by average hours per trip to yield average number of turtles per trip as follows.

Table 10. Estimated average turtles per commercial shrimp trawler trip for SC (12 hours per trip).

| | June | July |
|-------------|-------------|-------------|
| 2001 | 4.98 | 6.70 |
| 2002 | 4.30 | 4.45 |
| 2003 | 6.42 | 3.88 |

The total turtle/shrimp trawler interactions for South Carolina were calculated by multiplying turtles per trip by the estimated total number of trips.

Table 11. Total number of reported trips per month in South Carolina

| | June | July |
|-------------|-------------|-------------|
| 2001 | 496 | 1,268 |
| 2002 | 1,126 | 831 |
| 2003 | 1,057 | 1,041 |

Table 12. Estimated Total Number of Shrimp Trawler/Loggerhead Turtle Interactions for South Carolina.

| | June | July |
|-------------|-------------|-------------|
| 2001 | 2,470 | 8,498 |
| 2002 | 4,843 | 3,701 |
| 2003 | 6,788 | 4,039 |

Summary

The numerous assumptions associated with an analysis of this type make the calculations highly speculative. However, the numbers should, at minimum, accurately reflect the degree of magnitude of shrimp trawler/sea turtle interactions. This high level of interaction suggests that TEDs are indeed functioning well, and if TEDs were not mandated in shrimp trawls, total numbers of strandings would probably be much higher than those annually observed. Assuming the above calculations are somewhere near reality, and further assuming that May interactions are equal to June, and August interactions are considered $\frac{1}{4}$ of July, then the estimated total warm-weather season interactions for South Carolina were 15,562, 14,311, and 18,625 for 2001, 2002, and 2003, respectively.

Turtle Health

In conjunction with efforts to restore threatened or endangered marine turtles to historical population sizes, increased attention has been focused on assessing the health of these populations as well as their numerical abundance (Lutz and Dunbar-Cooper, 1987; Bolten and Bjorndal, 1992). In addition to providing an indication of the overall health of a wild population with time, determination of 'normal' values for free-ranging individuals also provides reference values to facilitate evaluation of the health of captive individuals being held for rehabilitation or educational purposes. Of a more immediate nature, health assessments have also been conducted to evaluate physiological stresses associated with collection and handling of marine turtles (Hoopes *et al.*, 2000), which is of particular importance as surveys to collect sea turtles in-water for the purpose of assessing population trends gain favor (Landry *et al.*, 1994).

Standard health assessments typically consist of qualitative evaluation of macroscopic condition and quantitative data derived from blood samples. Blood values are best utilized clinically over time to monitor changes in the physiological state of the animal. Alterations from the reference values may be due to a combination of factors that may vary temporally, spatially, and among individuals. Considerable efforts have been made to document the effects of season (Lutz and Dunbar-Cooper, 1987; Bolten and Bjorndal, 1992) and sampling (Bolten *et al.*, 1992) on clinical pathology values, in order to facilitate comparison of results among studies. Similarly, the effects of nutrition on selected clinical pathology values for sea turtles have also been examined (Moon *et al.*, 1999).

This component of the SCDNR in-water study presents data to supplement published reports on hematological values reported for wild and captive loggerhead sea turtles. The values presented were generated from opportunistic, one-time captures of wild loggerhead sea turtles and may reflect a variety of physiological conditions such as nutritional status, pathogen and parasite exposure/susceptibility, trauma, environmental conditions or a combination of these factors.

Methods

General Physical Exam

A general physical exam was conducted for each turtle. The shell, skin, muscle and flippers were routinely examined for trauma, epibionts, tumors, malnutrition, bite marks, missing or defective anatomical features, foreign bodies, sloughing of tissues, or the presence of residues (e.g., oil and tar). The skull (eyes, nares, oral cavity) was examined for discharge, corneal lesions, tumors, foreign bodies, and color of the mucous membrane (oral cavity). A general neurological test (visual and tactile stimuli) was conducted to evaluate responsiveness and coordination. A visual respiratory exam was included to evaluate breathing for excessively shallow (1 breath/min) or rapid (>5 breaths/min) breathing and to note the turtle's head position when breathing. Turtles were classified as "sick" when exhibiting at least two of the following conditions: general lethargy, emaciated/ poor body condition, old wounds that have not healed and/ or appear

infected, or a heavy load of epibionts (leeches, barnacles). By default, all non-sick turtles were classified as "healthy".

Blood collection, processing and analysis

Blood samples were used to evaluate sea turtle health using widely recognized diagnostic parameters. Three blood parameters (hematocrit, total proteins and glucose) were regularly determined aboard research vessels, while a fourth parameter (erythrocyte sedimentation rate, ESR) was only determined aboard research vessels for selected turtles during 2003. A professional diagnostic laboratory was contracted to analyze 27 blood parameters for a sub-set of sea turtles.

Sea turtles were placed in a restraining chair (head down, plastron flush to the chair) for collecting blood samples. Blood samples were collected from the dorsal cervical sinus of sea turtles, as described by Owens and Ruiz (1980). Betadine was applied to the needle insertion area prior to inserting a 21-ga Vacutainer needle with an associated plastic hub. An assistant(s) helped restrain each turtle while another person collected the blood sample, using one hand to stabilize the needle-hub apparatus and the second hand to switch out vacutainer tubes. A third person was responsible for inverting vials and handing empty vacutainer tubes to the blood collector, who processed the blood.

Blood glucose was determined using 1-2 drops of whole blood (5ml RT vacutainer tube) using a Prestige Smart System blood glucose meter (Home Diagnostics, Inc, Ft. Lauderdale, Florida, USA). The Prestige glucose meter measures whole blood glucose using membrane technology utilizing the glucose oxidase/ peroxidase reaction, and the results are colorimetric.

Hematocrit was determined by injecting a ~0.1 ml of whole blood into a micro-capillary tube, centrifuging for 5 min (14,000g, Model MB micro-capillary centrifuge, International Equipment Co., Needham Heights, Ma, 02490, USA) and then measured using a Lancer Critocap capillary tube reader.

Total proteins were determined using centrifuged plasma (5 min @ 1000g, Adams Sero-fuge CT1600, Clay-Adams Co., Parsippany, NJ, USA 07054); the plasma was extracted via pipette and placed on the refractive lens of a refractometer (RHC-200ATC, Westover Scientific, Woodinville, Wa, 98077, USA).

ESR was determined by gravity. A 1.6 ml blood sample was collected in a narrow diameter vacutainer tube, inverted 5x to ensure proper mixing of whole blood mixed with anticoagulant (sodium citrate), and then placed in a calibrated holding rack such that the bottom of the meniscus was aligned with the 'zero' mark on the rack. After 30 min, the position of the meniscus with respect to the scale on the holding rack was recorded.

Blood samples to determine Complete Blood Chemistry/CBC (Antech's Comprehensive Reptile Profile-AE160) profiles (Antech Diagnostic Laboratories, Memphis TN, 1-888-397-8378) were determined for turtles collected during the last 24 h of each weekly sampling cruise. Aboard research vessels, 5ml GT lithium heparin vacutainer tubes were inverted gently five times to insure proper mixing of blood and anti-coagulant; blood tubes were immediately placed upright in an ice bath and transported directly to the onboard lab. Blood smears were produced from whole (non-heparinized) blood, and 0.6 ml of whole blood was transferred to lithium heparin gel

microtainer, which was centrifuged (5 minutes @ 1000g) and refrigerated (along with residual whole blood tube and slide smears) prior to overnight shipping to Antech.

CBC samples were analyzed using the *Test Express* system to minimize variability in lab results by testing all samples using the same diagnostic machine (Hitachi 747-100), and utilizing the same technician for blood cell counts estimates. Potentiometric assays were used to determine sodium, potassium and chloride values; all other chemistry assays were photometric. Manual evaluation of blood smears by Antech technician was utilized to produce WBC and differential values. Hematocrit values were generated using microhematocrit tubes, centrifugation and manual evaluation of hematocrit tube.

Data analyses

Descriptive analyses were used to characterize most data. Percent occurrence of turtles observed with particular conditions as noted during the macroscopic health exam data was summarized graphically. A sub-set of loggerhead sea turtles for which Antech data were collected were characterized as either “sick” or “healthy”. Values for seven diagnostic parameters (glucose, hematocrit, total protein, BUN, Uric acid, WBC, CPK) were statistically compared among “sick” and “healthy” turtles using a Mann-Whitney non-parametric t-test. Descriptive statistics (mean, min, max, std. dev) for these seven Antech parameters were compared with published data. A descriptive summary (frequency distribution) of ESR data collected during 2003 was also included.

Results and Discussion

General Health Examination

Trawling efforts in 2000-2003 resulted in the collection of 946 loggerhead turtles. Approximately 3% of all loggerheads collected appeared emaciated or lethargic. Similarly, approximately 5% of all loggerheads collected had a large barnacle load. Physical trauma to the shell and/or flippers was noted for 5-13% of all loggerheads, with greatest frequency of occurrence involving damage to the carapace and flippers. Unusual external attachments (lesions, growths, leeches) or puncture wounds were typically observed in less than 2% of all loggerheads (Figure 25).

Trawling efforts in 2000-2003 resulted in the collection of 68 Kemp's ridleys. Unlike loggerheads, neither heavy biota load nor emaciated appearances were observed for any Kemp's ridleys. Similar trends as observed for loggerheads were noted for physical trauma among Kemp's ridleys, with greatest frequency of occurrence involving the carapace and flippers, however overall percent occurrence of these conditions was slightly lower in Kemp's ridleys than observed for loggerheads. Approximately 6% of all Kemp's ridley sea turtles were observed with tissue trauma to the tail and/or cloaca; in most instances, this trauma involved a prolapsed cloaca first noted upon removal from the net, which was typically resolved before these turtles were processed and released. Unlike loggerheads, unique external attachments (lesions, growth, leeches) were not found for any Kemp's ridley sea turtles (Figure 25).

Trawling efforts in 2000-2003 resulted in the collection of eight green sea turtles. None of the greens collected appeared to be sick or impaired by a heavy epibiont load. Physical trauma was only observed for three turtles, one collected in each of the fishery-

independent trawling regions. Unlike loggerheads and Kemp's ridleys, no physical trauma involving the carapace was observed among green sea turtles. Physical trauma involved slight abrasions to the flippers and the head/neck region, and although sample size was small, these minor conditions were observed frequently and may have been net related. No other noteworthy conditions were reported for green sea turtles.

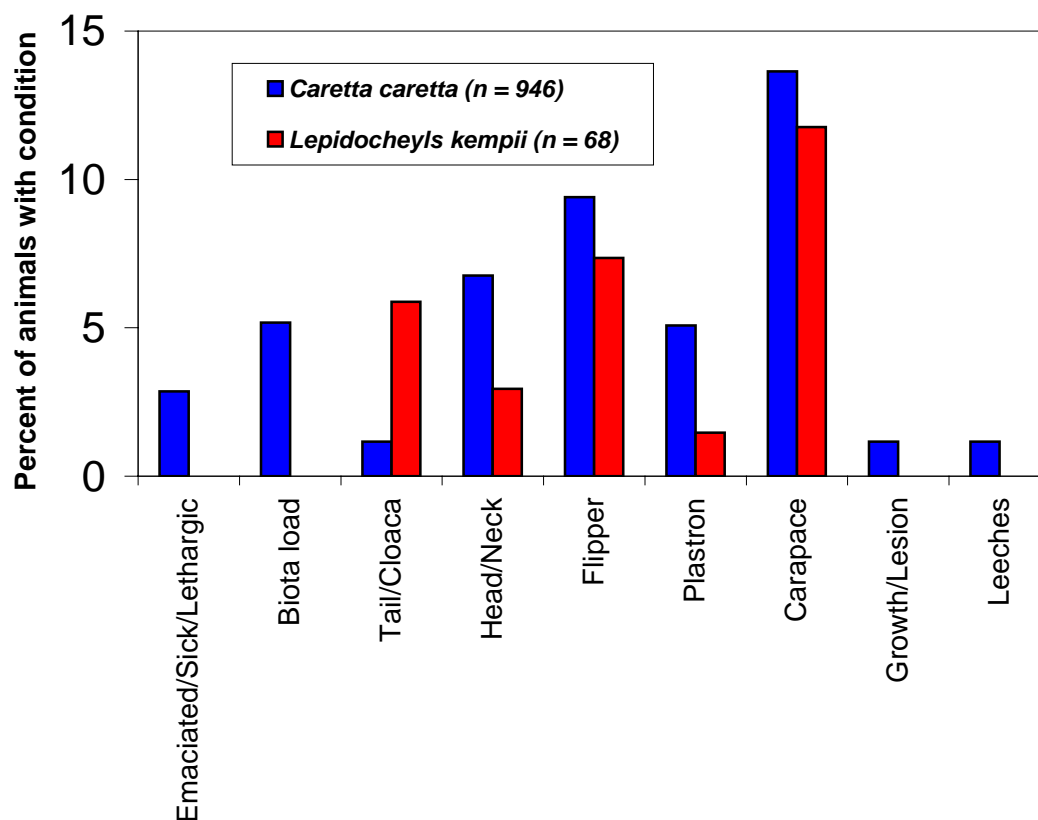


Figure 25. Summary of physical condition, excessive biotic load, and damage to specific anatomical regions.

Baseline Antech blood parameters for healthy turtles

A major health assessment objective was the identification of relevant blood parameters for assessing health of loggerhead turtles. Of 946 loggerhead and 68 Kemp's ridley sea turtles collected during 2000-2003, 172 loggerhead (18%) and six Kemp's ridley (9%) blood samples were processed by Antech. Of the loggerhead samples, 154 were from "healthy" turtles, eight were from "sick" turtles, and ten samples (<6%) were discounted for various reasons.

Descriptive statistics for all 27 Antech parameters for healthy and sick loggerhead sea turtles are presented in Table 13. Minimum straight-line carapace lengths measurements were available for 146 healthy loggerheads, of which 139 (95%) were 50-80 cm (24 M; 78F; 37 U). The remaining seven healthy loggerheads were 83-96 cm SCLmin (4M: 3F). Sick loggerheads measured 57-69 cm SCLmin (1M: 3F: 4U).

Table 13. Descriptive statistics for 27 blood parameters for healthy and sick loggerhead sea turtles.

| Blood Chemistry | Healthy | | | | | | Sick | | | | |
|------------------------|----------------|-------------|-----------|------------|------------|--|-------------|-------------|-----------|------------|------------|
| | n | Mean | SD | Min | Max | | n | Mean | SD | Min | Max |
| Albumin | 147 | 1.1 | 0.3 | 0.4 | 2.8 | | 8 | 0.6 | 0.4 | 0.8 | 0.2 |
| AST | 147 | 209.9 | 81.2 | 72.0 | 564.0 | | 8 | 194.8 | 118.0 | 271.0 | 45.4 |
| BUN | 146 | 78.9 | 26.9 | 16.0 | 150.0 | | 8 | 74.0 | 63.0 | 88.0 | 9.6 |
| Calcium | 147 | 7.8 | 1.5 | 1.6 | 11.7 | | 8 | 6.7 | 5.2 | 8.0 | 0.8 |
| Chloride | 147 | 117.5 | 7.4 | 92.0 | 141.0 | | 8 | 119.5 | 108.0 | 136.0 | 9.4 |
| CPK | 147 | 1235.3 | 1313.8 | 126.0 | 13830.0 | | 8 | 600.4 | 197.0 | 1208.0 | 365.3 |
| Globulin | 147 | 3.2 | 0.9 | 1.4 | 5.1 | | 8 | 1.9 | 1.2 | 3.2 | 0.7 |
| Glucose | 146 | 107.5 | 32.2 | 26.0 | 202.0 | | 8 | 74.0 | 45.0 | 112.0 | 20.7 |
| Phosphorous | 147 | 7.5 | 1.2 | 4.9 | 11.4 | | 8 | 7.0 | 5.9 | 7.9 | 0.7 |
| Potassium | 147 | 4.9 | 1.5 | 3.2 | 19.9 | | 8 | 4.5 | 3.7 | 5.3 | 0.6 |
| Sodium | 147 | 156.9 | 6.0 | 137.0 | 186.0 | | 8 | 155.3 | 146.0 | 162.0 | 5.0 |
| Total Protein | 147 | 4.3 | 1.0 | 1.8 | 6.6 | | 8 | 2.5 | 1.6 | 4.0 | 0.9 |
| Uric Acid | 147 | 1.6 | 0.7 | 0.1 | 4.0 | | 8 | 0.9 | 0.5 | 1.4 | 0.3 |

| Complete Blood Count | n | Mean | SD | Min | Max | | n | Mean | SD | Min | Max |
|-----------------------------|----------|-------------|-----------|------------|------------|--|----------|-------------|-----------|------------|------------|
| WBC | 153 | 11.1 | 4.0 | 4.0 | 25.0 | | 8 | 11.3 | 6.0 | 19.0 | 4.2 |
| Basophils | 153 | 0.2 | 0.6 | 0.0 | 3.0 | | 8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Eosinophils | 153 | 0.9 | 2.4 | 0.0 | 16.0 | | 8 | 0.6 | 0.0 | 2.0 | 0.9 |
| Heterophils | 153 | 35.5 | 18.1 | 7.0 | 86.0 | | 8 | 40.8 | 9.0 | 81.0 | 27.8 |
| Lymphocytes | 153 | 61.7 | 19.4 | 13.0 | 93.0 | | 8 | 57.0 | 15.0 | 91.0 | 29.7 |
| Monocytes | 153 | 1.1 | 1.5 | 0.0 | 7.0 | | 8 | 1.5 | 0.0 | 8.0 | 3.0 |
| Az Monocytes | 27 | 2.7 | 2.2 | 0.0 | 10.0 | | 3 | 0.3 | 0.0 | 1.0 | 0.6 |
| Absolute Basophils | 153 | 14.7 | 47.0 | 0.0 | 270.0 | | 8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Absolute Eosinophils | 153 | 80.6 | 212.0 | 0.0 | 1260.0 | | 8 | 52.5 | 0.0 | 180.0 | 75.5 |
| Absolute Heterophils | 153 | 7146.5 | 4067.7 | 1280.0 | 21000.0 | | 8 | 5147.5 | 1080.0 | 14440.0 | 5053.6 |
| Absolute Lymphocytes | 153 | 123.2 | 174.8 | 0.0 | 840.0 | | 8 | 5777.5 | 2100.0 | 10920.0 | 3152.9 |
| Absolute Monocytes | 153 | 3784.6 | 2472.3 | 700.0 | 22880.0 | | 8 | 260.0 | 0.0 | 1520.0 | 545.5 |
| Absolute Az Monocytes | 27 | 221.5 | 170.4 | 0.0 | 700.0 | | 3 | 33.3 | 0.0 | 100.0 | 57.7 |
| Pack Cell Volume | 104 | 34.9 | 4.1 | 21.0 | 45.0 | | 5 | 23.6 | 13.0 | 39.0 | 10.9 |

Clinical pathology of healthy vs. sick loggerheads

In addition to establishing baseline values for healthy loggerhead turtles, a second main objective of the clinical pathology portion of the in-water health assessment was to examine the magnitude of variation in values for specific parameters between and among healthy and sick loggerheads. Although analysis of 27 blood parameters is standard procedure for diagnostic laboratories such as Antech, a sub-set of seven parameters (hematocrit, total solids, blood glucose, blood urea nitrogen and creatine kinase) are consistently used for health assessments among veterinarians (George, pers. comm.; Harms, pers. comm.; Lewbart, pers. comm.; Norton, pers. comm.; Sheridan, pers. comm.)

and rehabilitation facilities (Topsail Sea Turtle Hospital, Volusia Marine Science Center) that specialize in sea turtles, other reptiles or other marine animals.

Descriptive statistics for seven standard clinical values for the 154 healthy and eight sick turtle values for which Antech data were available are presented in Table 14. Two sets of values were available for three parameters (hematocrit, total protein, glucose), which were measured both at-sea aboard research vessels using fresh samples and measured by Antech using 1-2 d old samples. Poor linear relationships were noted for all three of these parameters, with boat-derived values 6-13% higher than Antech values, on average (Table 15). General declines in these values with time may reflect true declines in concentrations associated with transport (i.e., red blood cell hemolysis), metabolic processes (proteins, glucose), differences in techniques used to calculate values, or interpretative differences among technicians recording values. Because all boat-derived values for hematocrit and total protein were observed within physiologically acceptable ranges (while some Antech values were not), boat-derived values for hematocrit were used in lieu of Antech-derived values in Table 14. Boat-derived blood glucose values were not substituted for Antech-derived values in Table 14 because Antech-derived values were determined using more sophisticated equipment than was available at sea.

Table 14. Descriptive statistics for selected clinical pathology parameters for “sick” (n=8) and “healthy” (n=154) loggerhead sea turtles. Boat-derived values were used for parameters denoted with an asterisk (*); all other values determined by Antech.

| Sick | Hematocrit* | TP* | CPK | Glucose | Uric Acid | WBC | BUN |
|-------------|--------------------|------------|------------|----------------|------------------|------------|------------|
| N | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| mean | 23.3 | 2.7 | 600.4 | 74 | 0.9 | 11.3 | 74 |
| stdev | 7.9 | 1.6 | 365.3 | 20.7 | 0.3 | 4.2 | 9.6 |
| min | 13 | 0.9 | 197 | 45 | 0.5 | 6 | 63 |
| max | 38 | 5 | 1208 | 112 | 1.4 | 19 | 88 |

| Healthy | Hematocrit* | TP* | CPK | Glucose | Uric Acid | WBC | BUN |
|----------------|--------------------|------------|------------|----------------|------------------|------------|------------|
| N | 153 | 153 | 146 | 146 | 147 | 153 | 146 |
| mean | 36.1 | 4.6 | 1149 | 107.5 | 1.6 | 11.1 | 78.9 |
| stdev | 4.9 | 1.1 | 797.8 | 32.2 | 0.7 | 4 | 26.9 |
| min | 23 | 1.8 | 126 | 26.0 | 0.1 | 4 | 16 |
| max | 57 | 7.4 | 4880 | 202.0 | 4 | 25 | 150 |

Table 15. Correlation between boat-derived and Antech-derived values of hematocrit, total protein, and blood glucose for individual turtles.

| | Hematocrit | Total Protein | Glucose |
|--------------------|-------------------------|------------------------|-------------------------|
| N | 113 | 156 | 72 |
| mean % diff | 5.8 | 6.7 | 12.6 |
| min % diff | -13.5 | -89.3 | -49.4 |
| max % diff | 84.6 | 88.9 | 728.6 |
| | | | |
| Correlation | $y = 0.5344x + 17.4170$ | $y = 0.2088x + 3.4982$ | $y = 0.5596x + 36.9780$ |
| R2 | 0.40 | 0.11 | 0.32 |

Detailed discussions of the values for these seven blood parameters for “sick” and “health” loggerhead turtles follow. Analyses were primarily descriptive in nature; however, formal statistical testing for differences was performed using the Mann-Whitney test, a nonparametric analog of the two-sample t-test (as unequal sample size and non-normal distribution did not satisfy the assumptions of ANOVA).

Hematocrit

Hematocrit is the percent of whole blood that is composed of red blood cells (MedlinePlus). Hematocrit values were statistically significant between healthy and sick turtles ($p < 0.001$), with higher mean values observed for healthy (36.1 %) turtles than for sick turtles (23.3 %). Similarly, standard deviation in hematocrit values for sick turtles was approximately 3x greater than the standard deviation observed for healthy turtles. Mean values for sick and healthy turtles were associated with near-extremes values considered as the normal (20-40) range of hematocrit in reptiles (Campbell, 1996).

A number of factors can be associated with low hematocrit, including acute blood loss, poor nutrition, chronic disease, parasites, and immune-deficiency (Duncan and Prasse, 1979). Although loggerheads with body conditions consistent with chronic poor nutrition were regularly observed, none of the sick loggerheads observed demonstrated evidence of acute blood loss; thus, chronic, rather than acute, factors were likely most responsible for depressed hematocrit values observed for sick turtles.

In contrast, hematocrit values may be elevated if turtles are dehydrated, which should also be reflected in an elevated total protein value due to hemoconcentration (Duncan and Prasse, 1979). One of the “sick” SCDNR animals demonstrated a PCV of 38% and a total protein value of 4.8 g/dl. Although this turtle was not considered for further evaluation and hospitalization, in retrospect, dehydration may have been responsible for the elevated hematocrit and total protein values determined at sea.

Further diagnostic tests (i.e., blood culture, fecal analysis, repeated CBCs, etc.) would be necessary to specifically identify the specific causative factors related to low hematocrit values in individual “sick” turtles. Given the low frequency of occurrence of sick” turtles in our samples, and the high individual variability in samples, additional testing to isolate causative factors is considered cost-prohibitive at this time.

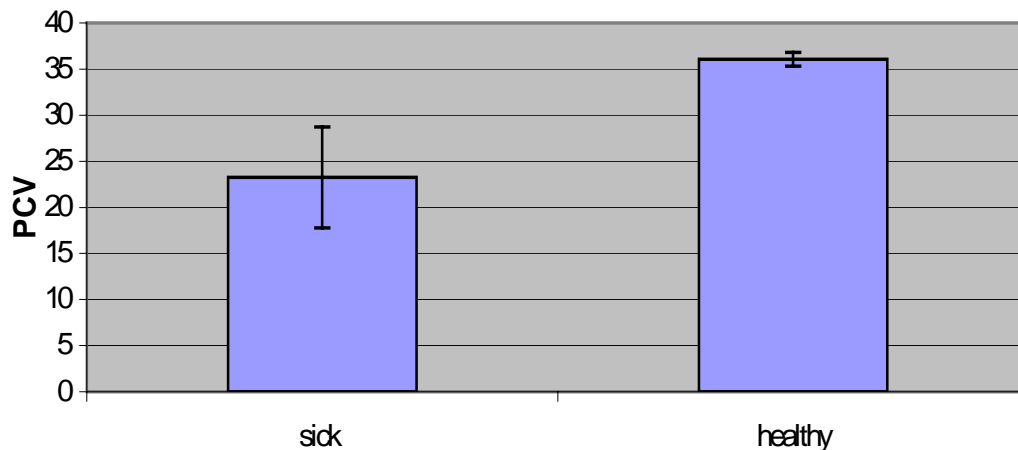


Figure 26. Mean and standard deviation for hematocrit values determined for sick and healthy loggerheads at sea. Significantly lower ($p < 0.001$) mean hematocrit values with a larger standard deviation were associated with sick loggerheads.

Total Protein

Total protein (TP) is a measure of proteins (albumin and globulins) in blood. Technically, the methods used to isolate total proteins in this study actually represent total solids; however, because of the low concentration of non-protein solids in plasma and the widely accepted use of refractive optometry to measure total solids, total solids are considered to be a surrogate value for total protein in this study.

Mean total protein value was significantly lower ($p = 0.002$) for “sick” turtles (2.7 g/dl) than for “healthy” turtles (4.6 g/dl). Similarly, standard deviation for total protein of sick turtles was approximately 4x greater than standard deviation for total protein of healthy turtles (Figure 27). Mean total protein value for sick turtles was slightly less than the generally accepted normal range of 3-8 g/dl for reptiles (Campbell, 1996). Mean healthy turtle TP was similar to the 5.5g/dl upper normal limit for total protein recognized by Antech Diagnostic Laboratories.

Hypoproteinemia in reptiles may result from a variety of influences, including malnutrition, parasitism, loss of protein enteropathies, severe blood loss and chronic hepatic or renal disease. Hyperproteinemia can occur with hemoconcentration (dehydration) or elevated globulins associated with chronic inflammatory disease (Campbell, 1996). Similar to hematocrit, hospitalization and rigorous study would be required to provide definitive explanation for depressed total protein values observed for sick turtles during this study, which is considered cost-prohibitive given the infrequent observation of sick turtles and the expense associated with additional testing.

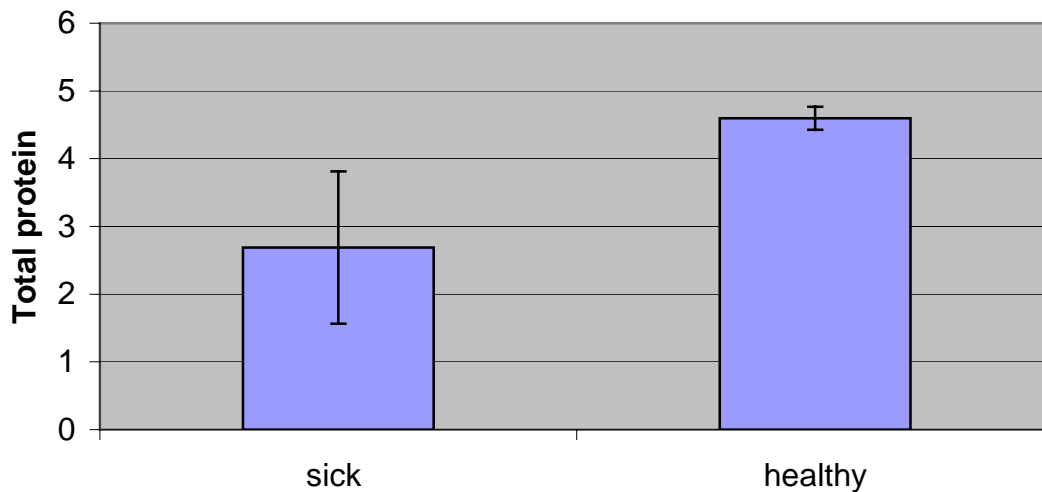


Figure 27. Mean and standard deviation for total protein determined for sick and healthy loggerheads at sea. Significantly lower ($p=0.002$) mean total protein values with a larger standard deviation were associated with sick loggerheads.

Glucose

Plasma glucose concentrations represent the availability of a critical energy source for metabolic processes. Mean glucose values were significantly lower ($p=0.003$) for sick loggerheads (74 mg/dl) than mean glucose values for healthy loggerheads (107 mg/dl) (Figure 28). Similar to hematocrit and total protein, greater standard deviation ($\sim 1.5x$) was observed for sick turtles than for healthy turtles. With the exception of a single healthy turtle with a glucose value of 7.0, mean, minimum and maximum glucose values for sick and healthy turtles were within the 40-120 mg/dl considered to be normal values by Antech Diagnostic Laboratories.

Hypoglycemia in reptiles can result from malnutrition, septicemia, endocrine disorders and severe hepatopathies (Campbell, 1996). The lowest glucose value for a healthy turtle was 25 mg/dl, which is well below the minimum 'normal' value specified by Antech. Sick turtles may have been stressed prior to capture and unable to mount a complete stress response minimizing temporary glucose elevation during capture. Sick turtles collected during this study may have been malnourished or suffering from sepsis; however, cost-prohibitive hospitalization and intensive diagnostics would be required to determine this.

Glucose levels for several sick turtles were above the lower limit of generally accepted 'normal' values. Higher than expected glucose levels for all turtles may have resulted from acute handling stress associated with capture (Duncan and Prasse, 1979). Similarly, higher than expected glucose levels may have been partially due to collection of sea turtles when surface water temperatures were $\geq 26^{\circ}\text{C}$. Mean plasma glucose levels in loggerheads have been documented to vary monthly by as much as 38 mg/dl, with a positively correlated relationship to water temperature (Bolten *et al.*, 1994).

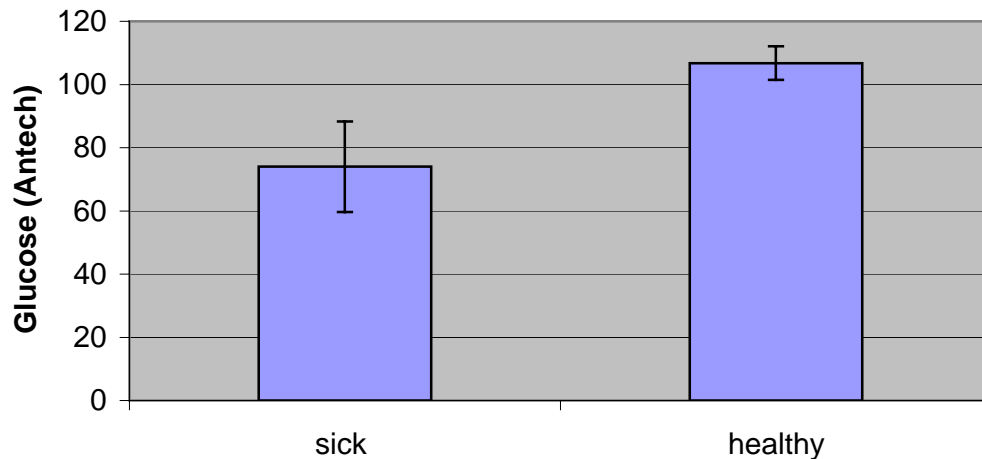


Figure 28. Mean and standard deviation for glucose determined for sick and healthy loggerheads at sea. Significantly lower ($p=0.003$) mean glucose values with a larger standard deviation were associated with sick loggerheads.

Creatine Kinase

Creatine kinase (CK, CPK) is an enzyme present in skeletal and smooth muscle of the myocardium, the gastrointestinal tract, and several organs (uterus, bladder, kidney and brain). In humans, CPK is used to evaluate the potential for muscle or brain damage in humans with myocardial infarction, as enzyme levels rise 4-8 h following a heart attack, returning to normal levels within 48 h (LabCorp). Despite the importance of monitoring this enzyme in humans, Antech Diagnostic Laboratories do not recognize a normal CPK range in reptiles.

Mean CPK values were significantly lower ($p=0.012$) in sick loggerheads (600 $\mu\text{g}/\text{dl}$) than mean CPK values for healthy loggerheads (1149 $\mu\text{g}/\text{dl}$) (Figure 29). Similar to hematocrit, total protein and glucose, standard deviation was approximately 2x greater for sick turtles than for healthy turtles. Without intensive diagnostic workup to include tissue and muscle biopsies, low CPK values observed for sick turtles during this study can only be assumed to have resulted from a lack of muscle mass due to a chronic disease process. Support for this assumption is based on low CPK values which are often encountered in elderly humans which generally have reduced muscle mass compared to younger adults (Labcorp) and in a form of Muscular Dystrophy (MD) found in children known as Duchenne muscular dystrophy, during which less CPK becomes available to muscles as the disease progresses and muscle mass deteriorates (MDAUSA).

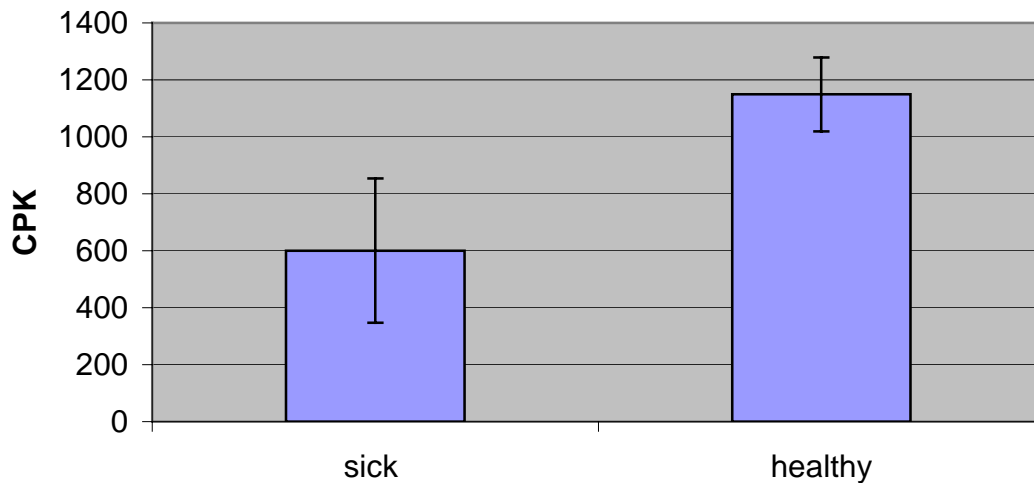


Figure 29. Mean and standard deviation for CPK determined for sick and healthy loggerheads at sea. Significantly lower ($p=0.003$) mean CPK values with a larger standard deviation were associated with sick loggerheads.

White Blood Cells

White blood cells fight infection by attacking foreign material that enters the blood stream (Nordenson, 2003). White blood cell (WBC) counts determine the total number of white blood cells and the percentage of each type of white blood cell in an animal's blood, providing insight regarding the presence of illness (i.e., increased white blood cell counts are associated with illness and immuno-suppression).

No significant differences ($p=0.502$) were noted between sick (mean = 11.3 THDS/CMM) and healthy (mean = 11.1 THDS/CMM) loggerhead sea turtles; however, standard deviation associated with sick turtles was approximately 4x greater than standard deviation associated with healthy turtles (Figure 30). WBC values were manually determined by blood smears evaluation, and caution should be exercised with regards to interpretation of these reported values. Because cellular responses of reptiles are less predictable than those of endothermic mammals and birds, the possibility of errors resulting from improper cell type differentiation is potentially high (Campbell, 1996).

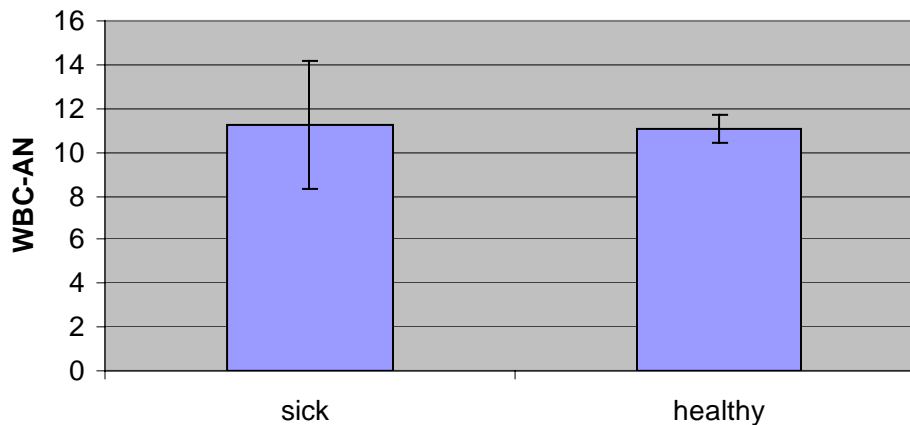


Figure 30. White Blood Cell (WBC) counts for sick and healthy loggerhead sea turtles. No significant differences were noted ($p=0.502$) among sick and healthy turtles.

Uric Acid

Uric acid is the primary catabolic end product of protein, nonprotein and purine in reptiles (Campbell, 1996). In humans, an excess of uric acid can cause gout (MEDLINE). In reptiles, loss of the majority of functional renal mass is required to elevate blood uric acid levels; thus, uric acid is not considered a sensitive indicator of reptilian renal disease.

Antech Diagnostic Laboratories recognize normal levels of uric acid in reptiles as 2-7 mg/dl. Mean uric acid levels were significantly lower ($p<0.001$) in sick turtles (0.9 mg/dl) than in healthy turtles (1.6 mg/dl) (Figure 31). Uric acid levels were lower than the lower 'normal' limit stated by Antech for both sick and healthy turtles. Sick turtles were associated with a larger standard deviation, approximately 2x the standard deviation associated with healthy turtles, which may have been related to small sample size.

In reptiles, diet effects blood levels of uric acid such that carnivorous animals tend to have higher uric acid levels than herbivorous animals (Campbell, 1996). In light of this information, opportunistically carnivorous loggerheads would be expected to have elevated uric acid levels, yet this was not the case for either healthy or sick turtles observed in this study. Given these observations, low uric acid levels may indicate poor nutrition due to foraging habits of loggerheads in the study area; however, more detailed information relating diet to foraging patterns would be necessary before any conclusion regarding this matter could be reached. Opportunistic monitoring of foraging habits of loggerheads in Chesapeake Bay over the past 20+ years indicates a shift in diet from horseshoe crabs to blue crabs to fish as the availability of earlier prey items diminished with time (Seney and Musick, 2004); thus, the possibility of the suggested scenario exists and warrants further investigation.

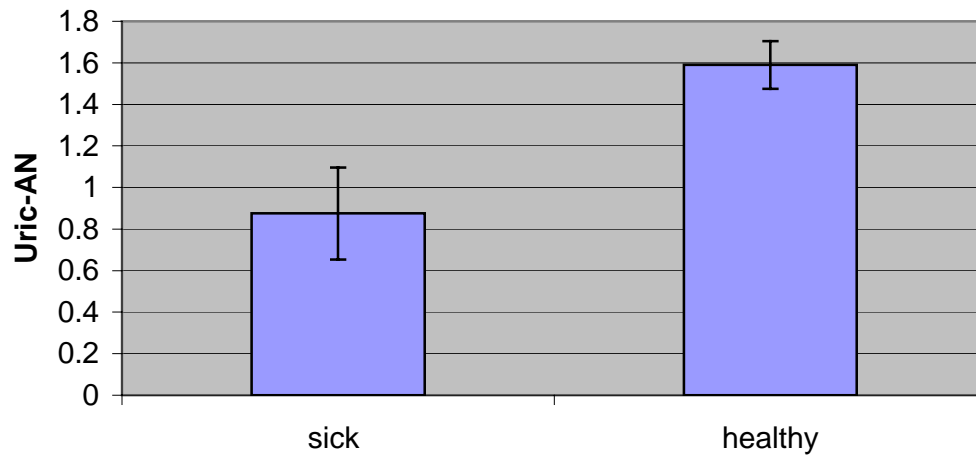


Figure 31. Mean and standard deviation for Uric acid determined for sick and healthy loggerheads at sea. Significantly lower ($p < 0.001$) mean Uric acid values with a larger standard deviation were associated with sick loggerheads.

Blood Urea Nitrogen

Blood urea nitrogen (BUN) measures urea nitrogen that forms as a result of protein metabolism. In mammals, BUN is often utilized to evaluate kidney function and may indicate poor nutritional status (MEDLINE). BUN is generally considered a poor diagnostic test for renal function in reptiles as uric acid is the primary catabolic end-product of protein (Campbell, 1996); however, BUN can be utilized as an indicator of nutritional status upon capture and during rehabilitation (pers. obs; George, pers. comm.).

No statistical differences ($p = 0.610$) were noted for mean BUN values between sick (74.0 mg/dl) and healthy (78.9 mg/dl) loggerhead sea turtles (Figure 32). Mean values for both sick and healthy turtles were elevated well above the 'normal' range of 1-30 mg/dl specified by Antech Diagnostic Laboratories, which calls into question the legitimacy of this range.

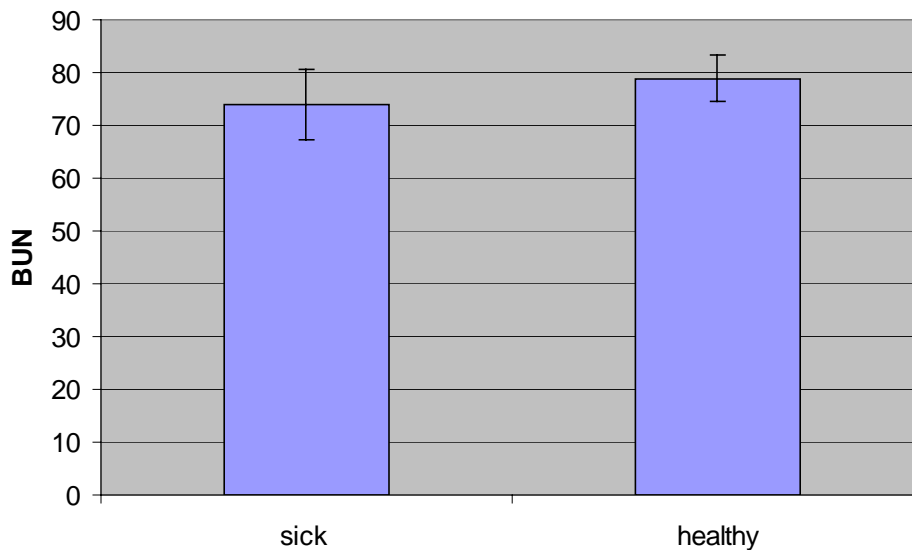


Figure 32. Mean and standard deviation for BUN determined for sick and healthy loggerheads at sea. No significant differences were noted ($p=0.610$) among sick and healthy turtles.

Comparison of clinical pathology parameters with published data

Descriptive statistics (count, mean, min, max, std. dev) for seven Antech CBC parameters (blood urea nitrogen, creatine phosphate kinase, glucose, hematocrit, total solids, uric acid and white blood cell count) for healthy loggerhead turtles were compared to similar data from previous studies. Blood chemistry values (mean, std. dev.) for several parameters from two monthly sea turtle trawl surveys in Cape Canaveral, FL (Lutz and Dunbar-Cooper, 1987; Bolten *et al.*, 1994) were averaged for the months of June and July 1980 and 1992. These data were also compared to similar data for sick or injured animals (International Species Information System, ISIS; cold-stunned turtles during the winter of 1999-2000 rehabilitated at the New England Aquarium, NEAQ 2000).

These blood parameters were compared with previously published loggerhead blood values in the following charts. With the exception of blood urea nitrogen (Figure 33), data for selected Antech parameters were similar among healthy loggerhead sea turtles collected during the 2000-2003 study and loggerheads collected by trawl from Cape Canaveral, FL, during June-July 1980 and/or 1992. Blood chemistry data for all trawl-caught loggerheads were generally more similar to Antech Diagnostic Laboratories “normal” values for these parameters than were data for aquarium or rehabilitated turtles (Figure 33-Figure 39).

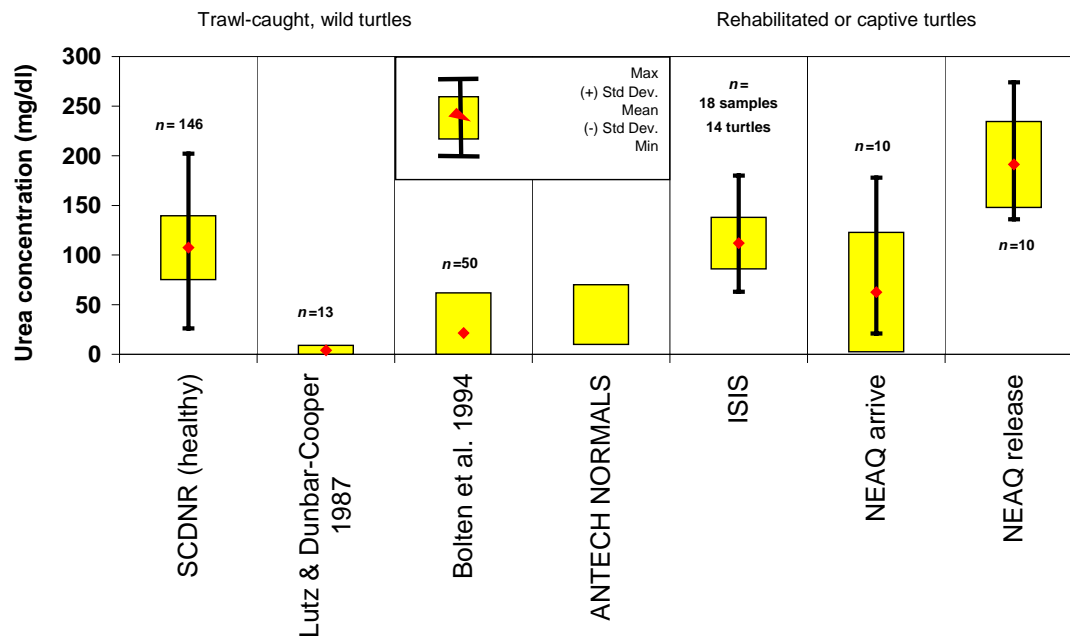


Figure 33. Comparison of blood urea nitrogen of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

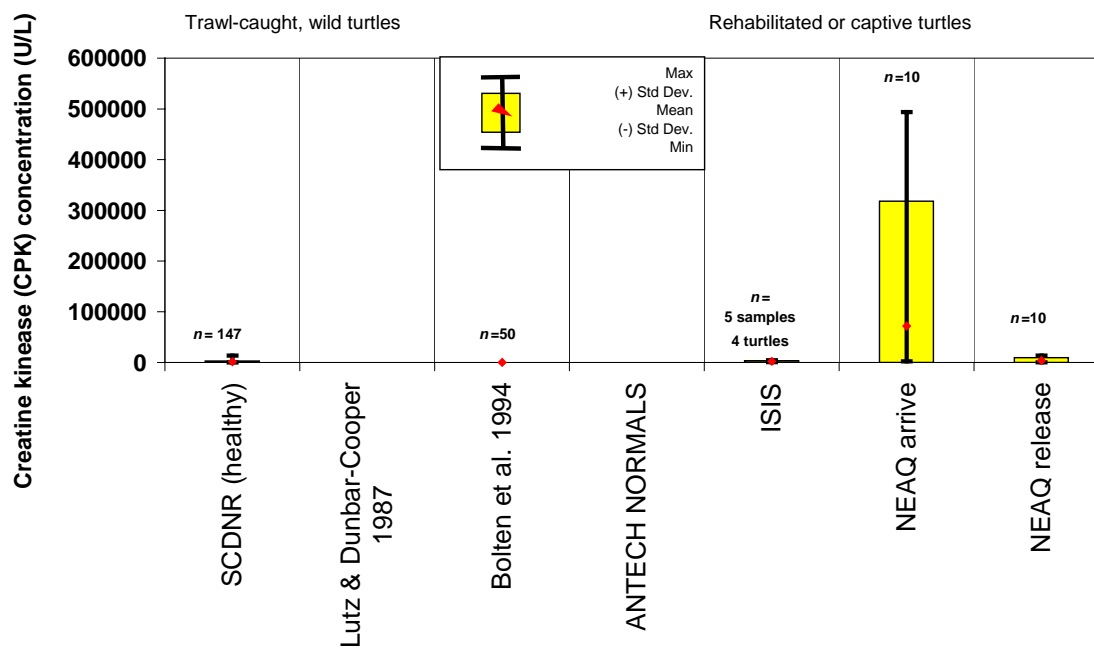


Figure 34. Comparison of creatine phosphate kinase of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

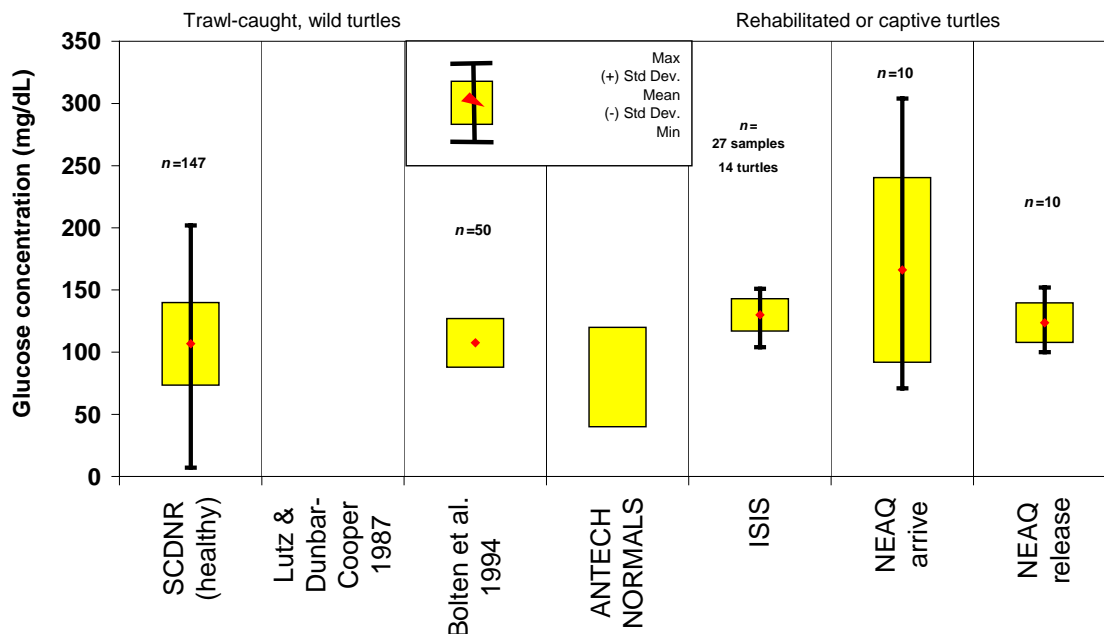


Figure 35. Comparison of blood glucose of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

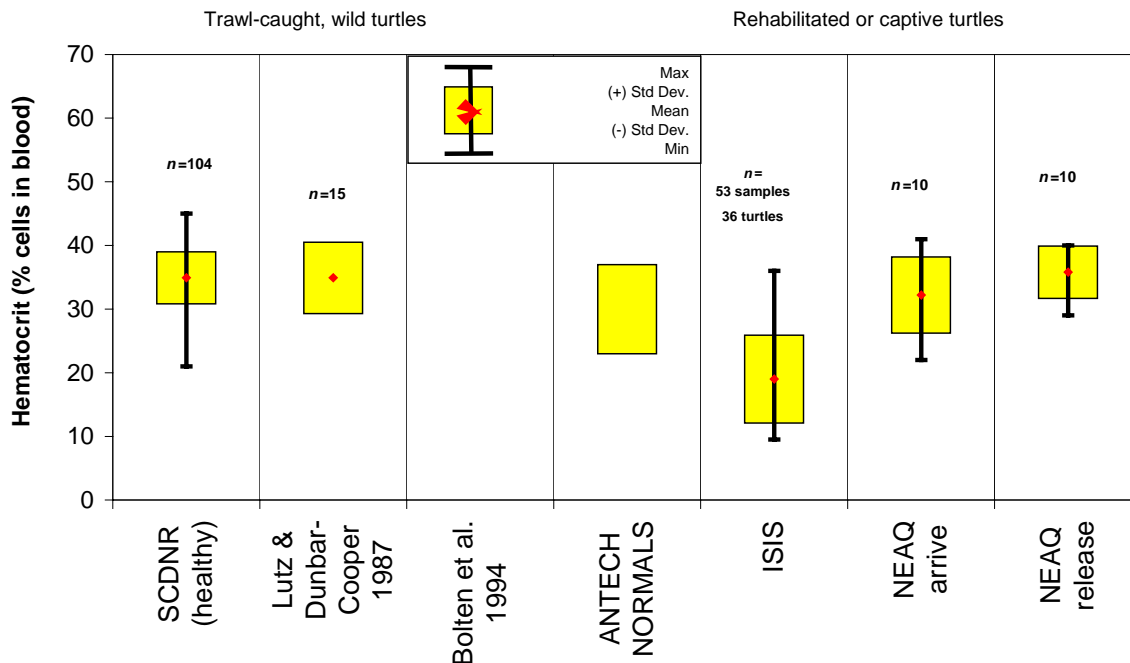


Figure 36. Comparison of hematocrit of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

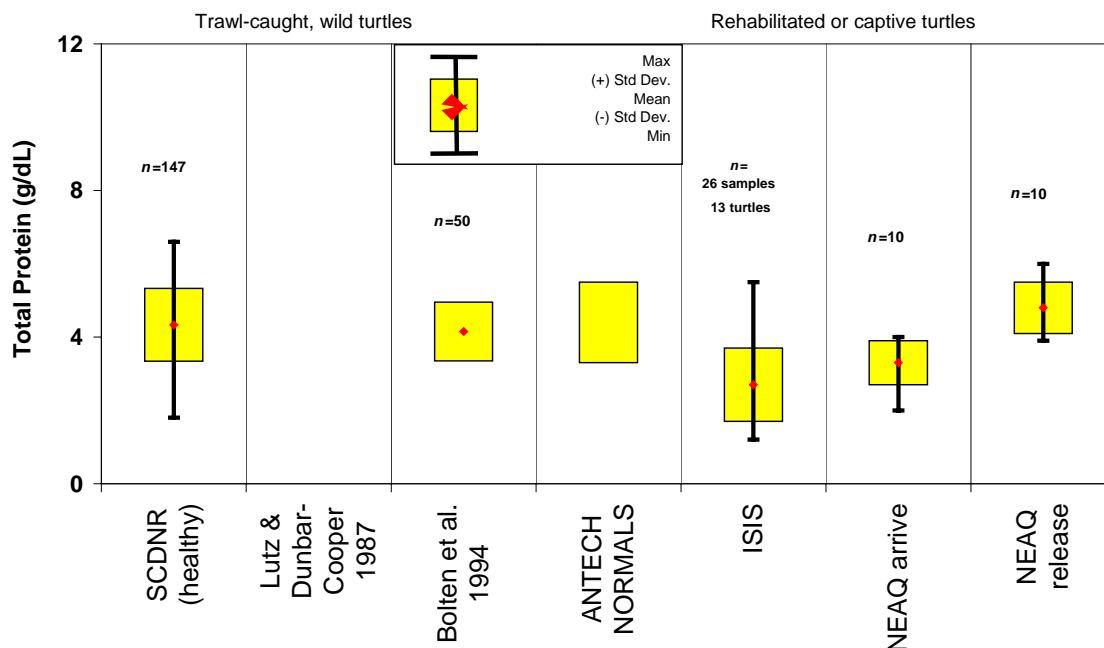


Figure 37. Comparison of total protein of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

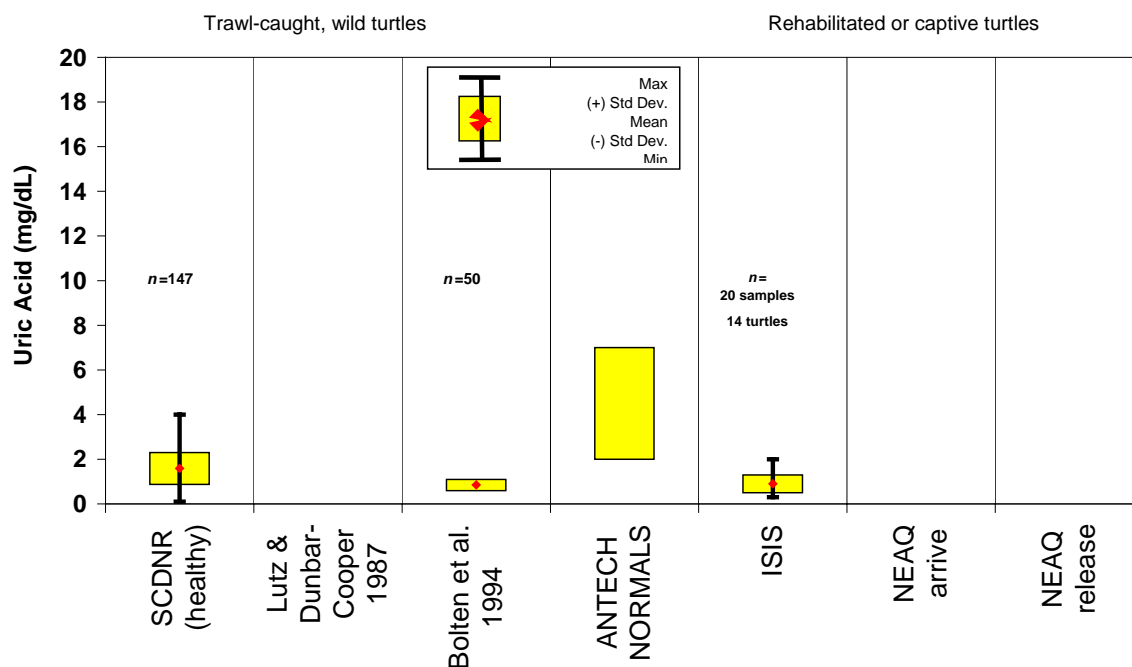


Figure 38. Comparison of uric acid of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

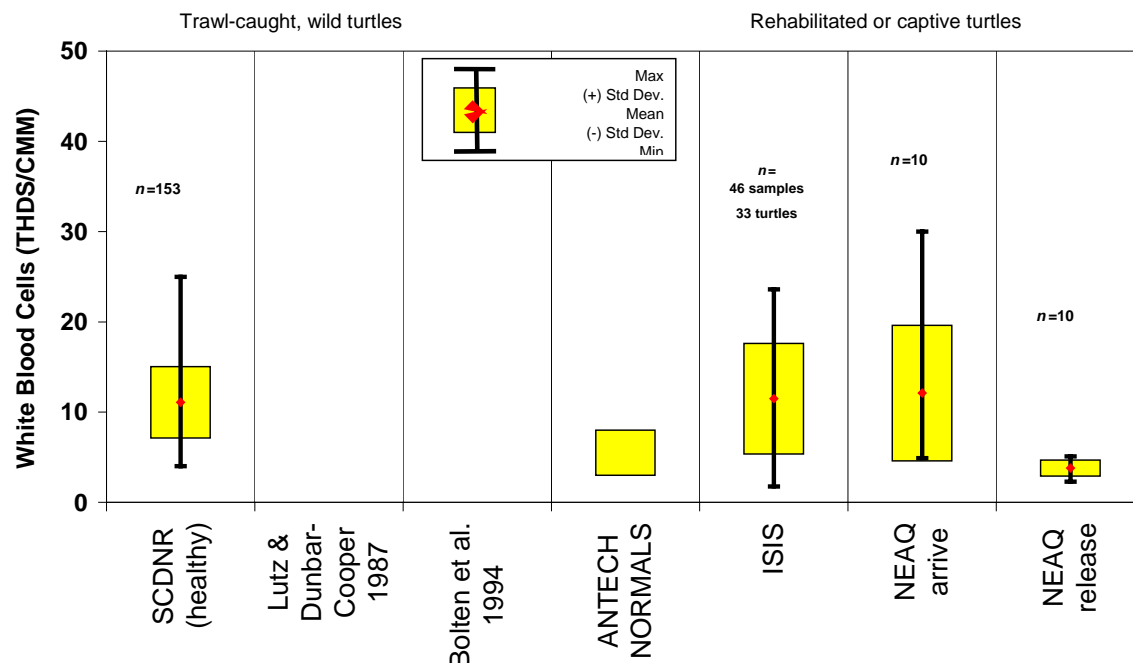


Figure 39. Comparison of white blood cell counts of healthy, trawl-caught loggerheads (2000-2003) versus trawl-caught and captive/rehabilitated turtles.

Erythrocyte Sedimentation Rate (ESR)

ESR is a reaction that measures the presence and intensity of inflammation in humans and some domestic animals. In healthy animals, red blood cells do not settle much during the course of the test; however, in sick animals, many disease processes stimulate formation of extra or abnormal proteins that bind to the red blood cells (rouleaux), resulting in clusters of cells that are heavier, fall faster, and result in a higher ESR score.

ESR is referred to as an acute-phase reactant test, meaning that it reacts to acute conditions in the body, such as infection or trauma. An advanced ESR value does not diagnose a specific disease, but does indicate that an underlying disease may be present. In human medicine, ESR is used to monitor a person with a known disease in order to track the progression of this disease, as ESR values will continue to increase as the effects of the disease increase (and the converse is also true). In bottlenose dolphins, ESR has been observed to increase during the acute phase of an inflammatory response or tissue injury, presumably due to increases in blood fibrinogen levels; thus, ESR is used often used as a prognostic tool (Bossart and Dierauf, 1990). ESR generally increases with elevated body temperatures or white blood cell counts. The ESR usually peaks after several days and usually lasts longer than the elevated temperature or white blood count (Bridgen, 1999; Nordensen, 1999; Medline Plus).

During summer 2003, a pilot study to obtain baseline ESR values for loggerhead sea turtles was initiated in cooperation with Greiner Bio-One of North America. ESR

values for 158 loggerheads and 15 Kemp's ridley sea turtles were obtained throughout the geographic area encompassed by the in-water sea turtle trawl survey. Higher mean ESR values were observed for Kemp's ridley (6.9) than for loggerheads (5.6); however, the range in loggerhead ESR values (1-15) occurred over a higher range than observed for Kemp's ridley sea turtles (3-10); and these differences may have been due to sample size (Figure 40).

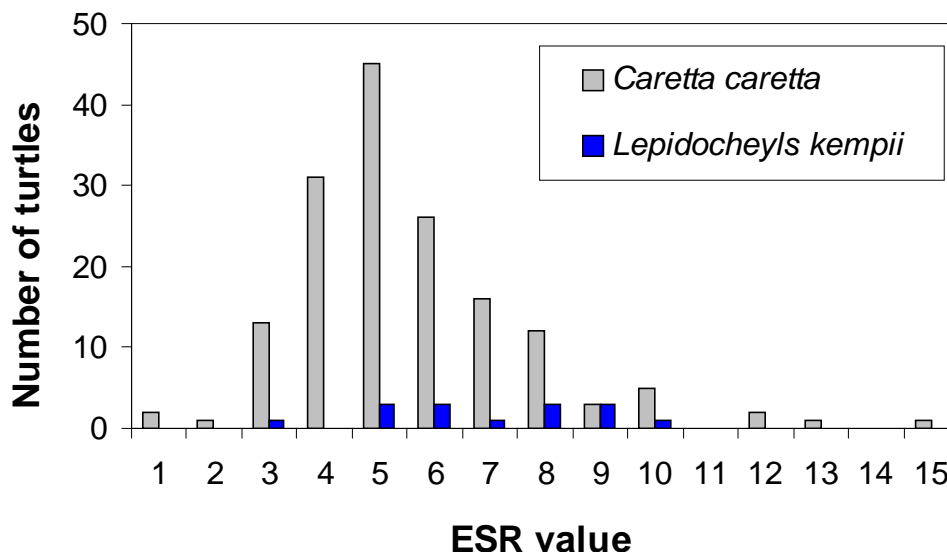


Figure 40. ESR values for 158 loggerheads and 15 Kemp's ridley turtles.

ESR values for four of the eight “sick” loggerheads referred to throughout the health assessment section of this report were available. All but one of these turtles (ESR=5,7,10 and 12) had ESR values higher than the mean for all loggerheads, and one turtle represented the highest ESR value recorded for loggerheads. Continued effort is being dedicated the ESR aspect of the project as stranded and recovering loggerheads are being tested to compare with the normal values developed in field work of summer 2003. Further analysis of this diagnostic tool will be done when data is developed from stranded and rehabilitated animals.

Summary

General health assessments were conducted for all sea turtles collected by trawling, to include a macroscopic inspection of body condition and documentation of several standard clinical pathological parameters. Overall, sea turtles collected during this in-water survey appeared to be in good health. Obviously sick or emaciated turtles comprised a very small percentage of the total turtles collected. Approximately half of all turtles collected possessed signs indicative of chronic physical trauma to either the hard body parts (carapace, plastron) or the extremities (flippers, tail, head/neck). The suspected cause of many of these injuries was attributed to boat strike events or possible predator interactions. Although such injuries (to include several turtles missing entire limbs) were regularly observed, blood chemistries did not suggest compromised health complications, corroborating anecdotal reports of the hardiness of sea turtles.

The underlying causes of sick conditions observed could not be determined, as numerous factors (i.e., cold winter, parasites, nutrition) interact to cause illness. Dehydration (hemoconcentration) was suspected in some instances, based on elevated hematocrit and total protein values. In addition to the absence of blood-borne parasites in all Antech samples analyzed, fecal parasites were also not detected in a handful of loggerhead samples analyzed (Greiner, pers. comm.). Poor nutrition may have contributed to the weakened conditions observed; however, further examination of the relationship between foraging habits of sea turtles and subsequent health are needed. Given foraging trends observed in the lower Chesapeake Bay (Seney and Musick, 2004) since the 1980's towards a potentially less nutritious diet (i.e., consumption of higher trophic level organisms), further examination of this relationship is recommended.

Complete diagnostic clinical pathology examinations were conducted for a subset of loggerhead sea turtles collected during this survey. Mean values for five of seven standard diagnostic parameters were significantly lower in sick sea turtles than for healthy turtles. Furthermore, standard deviations for these parameters were generally 2-4x greater for sick turtles than for healthy turtles; however, small sample size of sick turtles ($n=8$) may have contributed to this observation. In most instances, values for both sick and healthy turtles were within the accepted 'normal' ranges of values for parameters; however, in several instances, observed values were well above or well below these 'normal' values. Given that historically accepted 'normal' values for sea turtles are frequently determined from captive or rehabilitated animals, the data collected during this study demonstrate a need to re-evaluate the normal ranges for several parameters as they pertain to free-ranging, healthy animals.

Blood analyte data collected for free-ranging loggerhead sea turtles during this study were generally consistent with data collected in central Florida during the early 1980's and 1990's (Lutz and Dunbar-Cooper, 1987; Bolten *et al.*, 1994). In addition to expanding the temporal and spatial range over which such data have been collected, the data collected in this study increase the number of samples collected during the summer months by several orders of magnitude. Furthermore, the geographic and temporal scope of such studies continues to expand (Kimmel *et al.*, 2004), and collaborative efforts to synthesize and thoroughly analyze existing data should be pursued in the near future.

We find it noteworthy that turtles evaluated as "sick" in this study were relatively small compared to the size distribution of the healthy turtles. Are small turtles more susceptible to pathogens or toxic pollutants that could compromise their health. Unfortunately, the small sample size does not allow us to adequately evaluate this potential phenomenon, but in the future researchers should be mindful of this possibility.

In addition to infrequent observation of sick turtles during this study, turtles that were sick enough to warrant shore-based attention were almost never observed. Between 2000-2003, only three turtles were ever transported to shore for rehabilitation. Two emaciated and lethargic sea turtles were selected for rehabilitation after being collected in a comatose state and showing signs of impairment following successful intubation. Both of these sea turtles were ultimately euthanized. Conversely, a sea turtle with a severely and recently damaged rear carapace and plastron was transported to Sea World for rehabilitation and was released less than four months later. These anecdotes underscore the hardiness of sea turtles to survive and prosper following physical trauma as well as the lessened ability of sea turtles to survive chronic health problems.

Inter-annual trends in sea turtle health were not examined in this section; however, future analyses of these data should investigate this aspect of sea turtle health trends. During 2003, tissue biopsy samples from two turtles collected in coastal waters of south-central GA were positively determined to be fibropapilloma (FP). These tumors were much smaller than tumors associated with green sea turtles and hawksbills from southern FL waters, but the timing (fourth year of the study) of occurrence suggests that this disease may be spreading northward, although FP has been reported at least as far north as NC as early as 2001 (Harms *et al.*, 2004).

This study relied on the collection of single-point-in-time blood samples to assess blood and biochemical values, which is not particularly useful regarding the specific etiology of the variation from normal of any individual animal. However, the information obtained during this study does strengthen the data available for health assessment evaluation in loggerhead sea turtles.

In addition to the paucity of existing clinical pathology data generated using large sample sizes, many of the analytical methods used by researchers lack standardization. Although hematologic complete blood counts (CBCs) are taken on ill turtles, these data are not as meaningful for turtles as they are for mammals and birds (Jacobson 1998). Hematocrit and white blood cell counts are highly regarded among researchers as good diagnostic parameters; however, these counts often lack correlation with specific diseases (Jacobson, 1998). These kinds of issues are further complicated by the fact that animals that appear perfectly normal may occasionally experience abnormal values (Rebar, 1999). While there are still improvements to be made with regard to assessing the health of wild and captive sea turtles, the data collected by this study during 2000-2003 provide an improved framework by which to assess sea turtle health, and additional contributions may result from future sea turtle health assessment efforts set to begin in 2004.

Collaboration and Outreach

Summary

From the inception of the project staff have placed a high value on the opportunity they were given to work with endangered and threatened sea turtles. This value was reflected in staffs' efforts to maximize data gathering and sharing at all stages of the project. Efforts included professional collaboration, public education through outreach and media involvement, and participation in formal education of students.

Professional Collaboration

During the project staff sought and received assistance from researchers at the local, regional, and national levels. Participation of individuals from government, educational and private institutions enriched the product we were able to produce. Table 16 identifies many of the individuals to whom we are grateful for their enthusiastic participation. Many of our professional collaborators have used samples we provided to further their own research interests or provide opportunities for training students.

Table 16. Summary of collaborative research efforts.

| <u>Collaborator(s)</u> | <u>Institution</u> | <u>Subject</u> |
|---------------------------|--|------------------------------|
| Dave Owens | Grice Marine Lab, College of Charleston | testosterone assay |
| Margie Peden-Adams | Medical University of South Carolina | immunology |
| Joe Quattro | University of South Carolina | genetics |
| Karen Burnett | Grice Marine Lab, College of Charleston | endocrinology |
| Ellis Greiner | University of Florida | parasitology |
| John Zardus | University of Hawaii | barnacle systematics |
| Craig Harms | North Carolina State University | hematology (ESR) |
| Jennifer Keller | Duke University, NIST | toxicology |
| Heather Wilson | University of Georgia | physiology (biliverdin) |
| Scott Weber | New England Aquarium | physiology (Vitamin D) |
| Amber Von Harten | Pritchards Island, U. of South Carolina | genetics |
| Dave Rostal | Wassaw Island, Georgia Southern University | genetics |
| Lucy Hawkes | Bald Head Island Conservancy | genetics |
| Kate Schaeffer | NOAA | offshore plankton assay |
| Terry Norton, Sharon Deem | Wildlife Conservation Society | sea turtle health assessment |

Public Education

Project personnel invested significant time and effort in this component of grant. Public education efforts have focused primarily on description and results of project itself, but have also incorporated the relevance of project to ecosystem monitoring and possible correlations to impacts on human health. Public presentations ranged in audience from 5th grade classes, civic clubs, non-governmental organizations to scientific presentations. Format of presentations was typically a 45-minute Powerpoint presentation. Depending on the audience the presentation was sometimes supplemented

with a 13-minute video of onboard turtle work-up produced by University of Georgia Marine Extension Service.

The five members of the project team gave dozens of public presentations. These ranged geographically from St. Augustine, Florida (Whitney Marine Lab) to Bald Head Island, NC. Scientific presentations related to this project have been made at a variety of venues from Sea Turtle Conferences (Philadelphia, Malaysia, Costa Rica) to regional scientific meetings (Skidaway Oceanographic Institute, Savannah, Georgia). Another effort to involve the public in this project has been the opportunity to "join scientists at sea". Day trips to join field work effort were offered to several organizations involved in marine education (South Carolina Marine Educators Association (3), Georgia Association of Marine Educators (2), Project Oceanica (2), South Carolina Aquarium (1), University of South Carolina- Beaufort/Pritchards Island Sea Turtle Project (4)). These organizations function to educate both the public as well other professional educators in the marine science realm.

Media

Representatives of media were invited to participate in project. Representatives from local and regional news organizations participated in cruises (Hilton Head- Island Packet (2 trips), Brunswick, Ga- Brunswick News, Charleston- Post and Courier, Columbia, The State. A number of the articles on project were syndicated nationally. Environmental Media Corporation (Beaufort, S.C.) is developing an educational series on loggerhead sea turtles; three representatives of Environmental Media joined cruise in '03.

Project Oceanica is developing information access on the project via internet based web site. This component of Project Oceanica serves educators, schools and the public as a source of information on research relating to marine science. Project Oceanica can be accessed via www.oceanica.cofc.edu/home.htm

Student Involvement

Veterinary students

Student involvement was deemed a priority and has been mutually beneficial to both our projects as well as the students themselves. Formal agreements were established with North Carolina State University and the University of Georgia Veterinary schools to involve interested students in fieldwork. In addition, veterinary students from Cornell University and the Royal Veterinary College in UK joined field efforts. Veterinary students became an integral part of the scientific field team and, following training, became valuable team members in collecting and processing data and samples. Three students from NCSU produced publications associated with the project (Barlett, blood gas response post-capture in loggerheads; Ross, Bonnethead sharks; Cain, sting rays). One University of Georgia student developed a website project describing her summer involvement aboard the R/V *Georgia Bulldog*.

Other students

Students from graduate, undergraduate, and high school and programs have taken advantage of the opportunity to join in project efforts (Table 17). Some have received funding assistance through sample processing associated with their graduate work, some

have received samples to support their research effort, while others have joined the project as paid scientific crew or even volunteers.

Table 17. Summary of students and institutions participating in the project.

| Institution | Number of Students |
|---|---------------------------|
| North Carolina State University School of Veterinary Medicine | 12 |
| University of Georgia | 7 |
| College of Charleston Grice Marine Biological laboratory | 4 |
| University of South Carolina | 2 |
| Duke University | 1 |
| Clemson University | 1 |
| George Washington University | 1 |
| Cornell University | 1 |
| Royal Veterinary College, United Kingdom | 1 |
| Winthrop University | 1 |
| Coastal Carolina University | 1 |
| Christsschool, Asheville, N.C. | 3 |

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